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GALILEO's genius for rapid solution of difficult problems is perfectly exemplified by his work with the telescope. Within several hours after hearing of the first telescope, he had mastered the principles involved. Within several months, he had made a scientific instrument of it.

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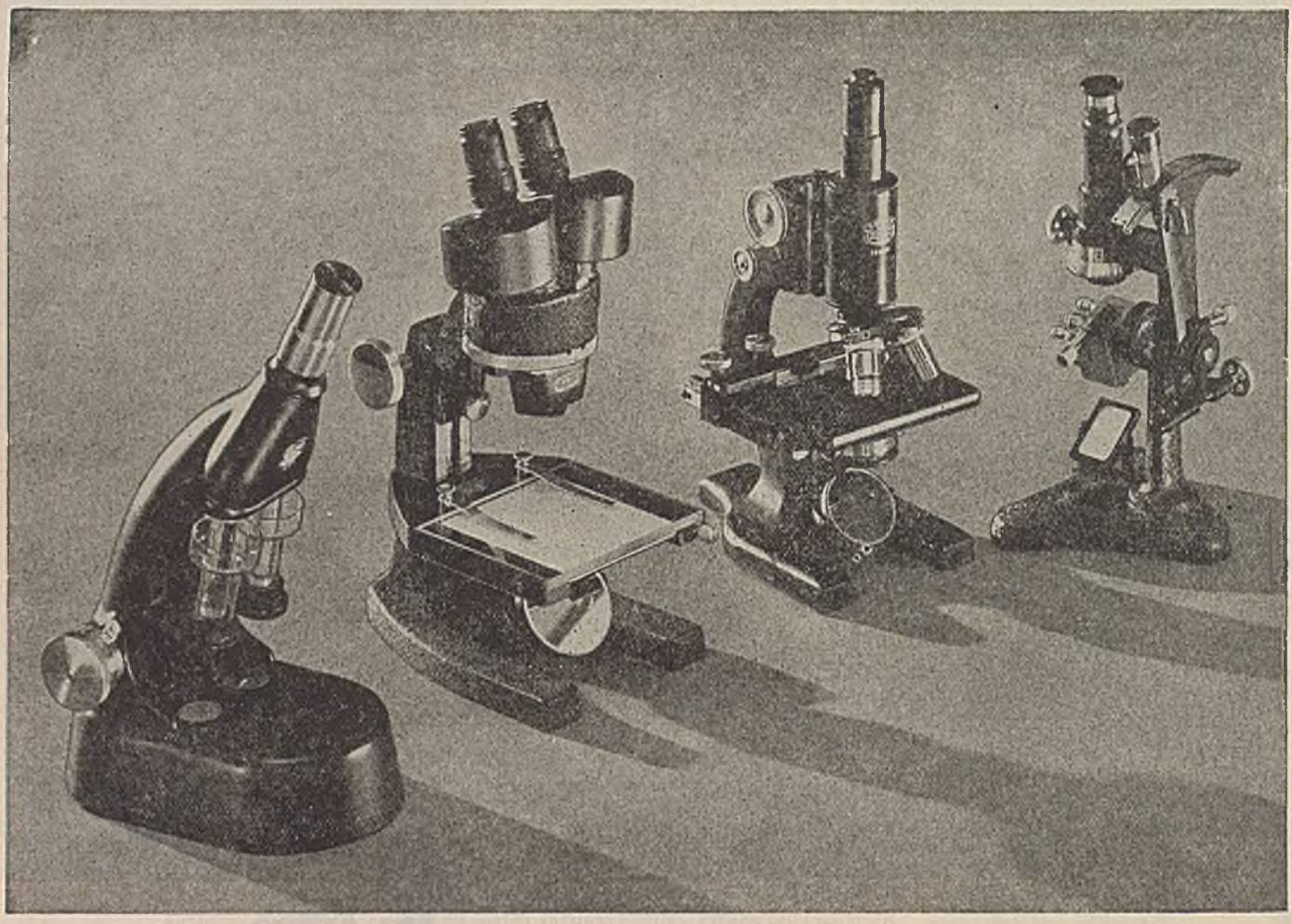

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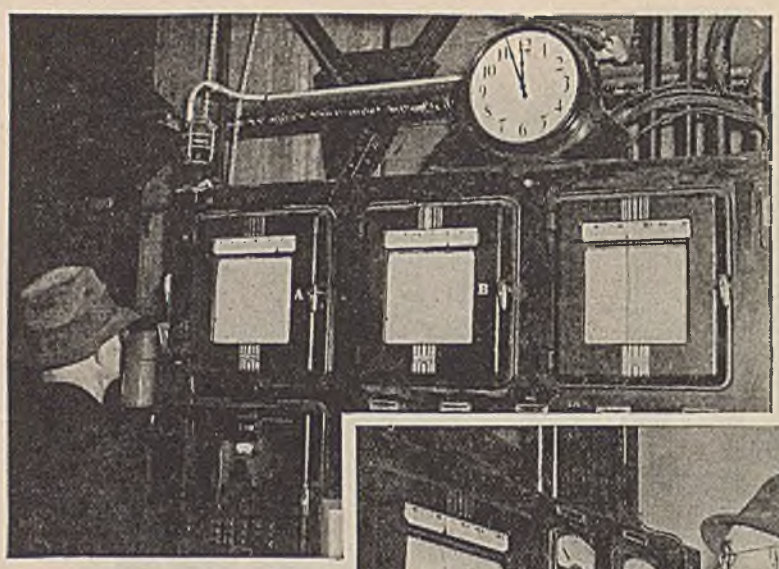

Above — Operator in Atlantic Refining Co.'s contact-process sulfuric acid plant, glances at Micromax Pyrometer Recorder to see trend of temperatures of SO_2. Recorder in center is also for temperature; one at right shows strength of finished acid. Its range is 96.5 to 99.5% H_2SO_4.

• • •

Right — Operator reads acid-strength Recorder and enters reading on process-control operations report.

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The most immediate evidence of successful plant operation is furnished by the acid-strength Recorder, shown in the lower photo. The strength is shown as 99.1% when photographed, and the record on its chart proves this strength had been held constant for the past 6½ hours. The straightness of the record proves smooth handling of all units on the acid line.

The two Temperature Recorders and the Indicator give the operator vital information when he is manipulating the push-buttons and other controls on the panels to secure the desired yield and strength of acid. The Recorders put down in curves the important temperature trends so he can see them at any time; the Indicator enables him to supplement these facts by reading the temperature of any thermocouple at any time.

These Instruments, together with Micromax SO_2 and pH Recorders, which are used in many acid plants, can be supplied as automatic controllers when desired. For details, please either ask for catalogs or for individualized facts, as you prefer.

How The Microphotometer Fits Into Radiation Work In Socony-Vacuum Labs

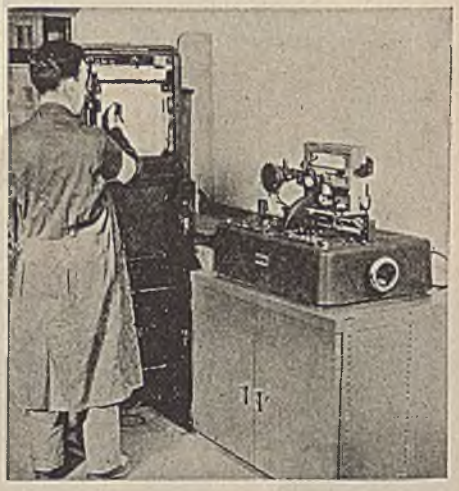
Following is an abstract from a paper "Radiation — The New Petroleum Analytical Tool" by Schlesman and Hochgesang of Socony-Vacuum Oil Co., in Jan. 13, '44, *Oil & Gas Jrl.* In the course of a lengthy report on the subject in general, they write:

"Quantitative spectrographic methods are especially useful to determine metallic elements in low concentration, where the accuracy of the spectrographic method usually exceeds other analytical methods. If a large number of analyses of a given kind are to be made, the spectrographic methods are (also) time-saving . . ."

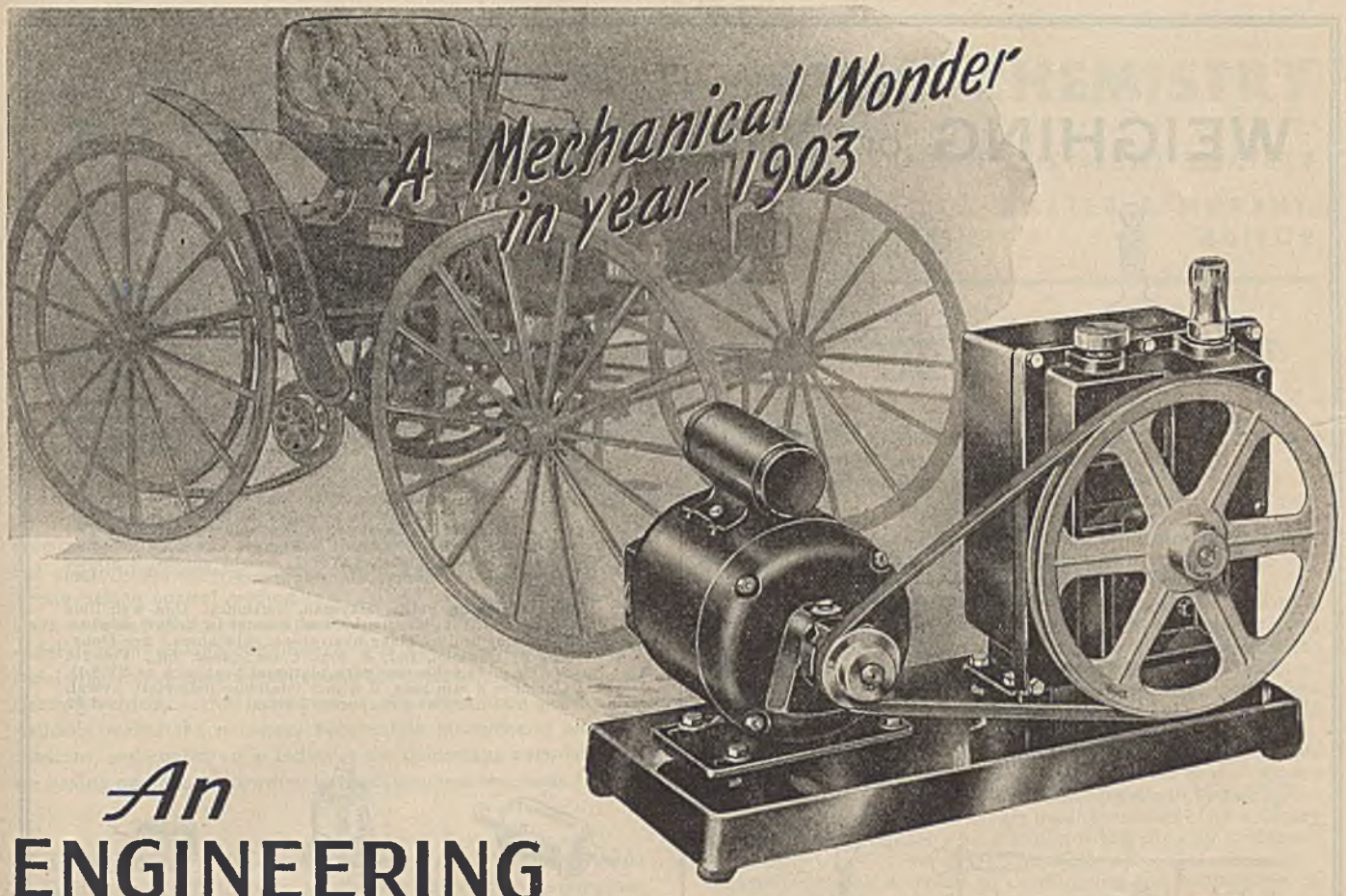
When using an L&N Knorr-Albers Microphotometer for this work, the authors say: "In analyzing for metals and metalloids, the sample is first burned, the spectrum recorded and the photograph developed under rigorously controlled conditions."

"The optical density of lines is automatically measured and recorded on a microphotometer, and the chosen lines are then compared with those from known samples of approximately the same composition. The method is empirical and procedures must be worked out for each analysis."

"If samples must be burned in a direct-current arc, the quantitative accuracy will probably not exceed ten per cent of the amount of the element present. For example, if a clay catalyst contained ten per cent magnesium, spectrographic methods would, at best, report it as 10%, plus or minus 1 per cent. But, if magnesium were present at 0.01%, it might be reported as 0.01% plus or minus 0.001%."



Chemist in Socony-Vacuum Oil Co.'s Radiation Lab., using an L&N Knorr-Albers Microphotometer as described herein. For a description of the instrument, ask for L&N Catalog E-90(1).



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INDUSTRIAL AND ENGINEERING CHEMISTRY
Analytical Edition
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A Needed Improvement in Baking Control Methods for Organic Finishes

STUART GRAVES, E. I. du Pont de Nemours & Co., Inc., Philadelphia, Pa.

Numerous variables, as discussed, introduce large errors in the control of short-time organic enamel baking operations. A relatively simple baking control method is described which compensates for these variables and gives comparable enamel film properties under widely varying baking conditions. It is based on enamel film or metal temperature determinations and the calculation of consequent reaction velocity. An instrument is also described which automatically makes the necessary temperature measurments and calculations, and continuously indicates the percentage completion of the baking operation, according to predetermined standards.

THE introduction during recent years of industrial organic finishes designed to bake at short, relatively high-temperature schedules emphasizes the need for more accurate methods of controlling baking operations. The long-established method of placing the metal to be baked in an oven, set at a given temperature, for a predetermined period of time gives satisfactory results when the baking time is in the order of one hour or longer. However, in the case of appreciably shorter bakes, for which higher temperatures are employed, serious variations are encountered in the enamel film temperature resulting from variables which are difficult or impossible to control. These variables include heating characteristics of the oven, mass of material in the oven, mass–surface area relationship of the painted ware, etc. They all have an effect on the baking speed of the enamel film and result in serious variations in the properties, such as color, hardness, water resistance, and durability of films baked according to predetermined time–oven temperature schedules.

Experimental evidence shows that the speed of curing or polymerization of an organic enamel film, like the speed of other chemical reactions, is a function of temperature. Furthermore, there is ample evidence to indicate that the temperature of an enamel film on a metalsubstrate follows very closely the temperature of the metal surface, regardless of the heat gradient between the metal and the surrounding air bath. The metal is a good conductor of heat and the air is a very poor one; hence any heat gradient at the paint film–metal interface is quickly neutralized because the heat is conducted away from or to the paint film by the metal. It follows that an accurate measurement of the surface temperature of the metal is also an indication of the temperature of the paint film. Such a measurement can be made with a contact thermocouple.

A copper-constantan thermocouple junction is brazed directly to the sample of ware to be baked, or, for the sake of simplicity it can be brazed to a square of thin sheet copper which is then clamped to the face of the painted metal sample in such a way that it is in intimate contact with the surface. A section of the sample is first wiped clean of the wet paint film. If the clamp is light in weight and if it is insulated from the metal with asbestos cloth, its added mass will not influence the temperature measurements appreciably, and any error by comparison with the former method of brazing directly to the metal object will be negligible. The temperature measurements are made by means of an ordinary laboratory potentiometer with a scale reading directly in degrees Fahrenheit.

Employing such a means of determining the temperature of the metal and of the paint film, a curve has been plotted (*A*, Figure 1) which shows the temperature-time relationship for a sample of metal in a laboratory box-type oven, the air temperature of which was controlled at 260° F. The sample consisted of 6 square feet of 26-gage sheet steel weighing approximately 4.5 pounds and painted on one side only. *B* represents the temperature rise for an equal area of painted 14-gage steel, weighing approximately 30 pounds, during a second run in the same oven. The correct baking time for the 26-gage metal to give paint film properties which have been arbitrarily set up as standards for the particular finish under consideration is 66 minutes. By inspection of the curve it is seen that, although the 14-gage metal lags somewhat behind the 26-gage for the first 20 or 30 minutes, it approaches very closely the temperature of the latter for at least 60 to 65% of the baking time. As a matter of fact, after 66 minutes of baking, the color, hardness, flexibility, etc., of the enamel film on the two metal samples are equal within the experimental limits of the testing methods.

This illustrates the fact that various weights of ware having varying thicknesses within reasonable limits can be baked for a predetermined period of time in the neighborhood of an hour or longer with little, if any, variation in resulting paint film properties. In shorter baking operations, however, this is not the case.

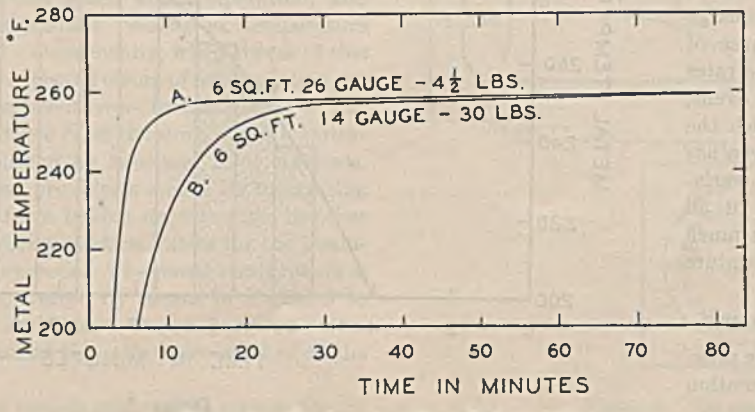

Figure 1. Temperature-Time Relationship

Curve *A*, Figure 2, shows the temperature rise for the 4.5 pounds of 26-gage steel and *B* represents that for the 14-gage in a 345° F. oven. The correct baking time for the light metal in this oven has been determined to be about 9 minutes. At the end of 9 minutes the heavier sheet, however, has reached only 285°F., and if it is removed from the oven at this time the enamel film is found to be soft, and to have poor water resistance and

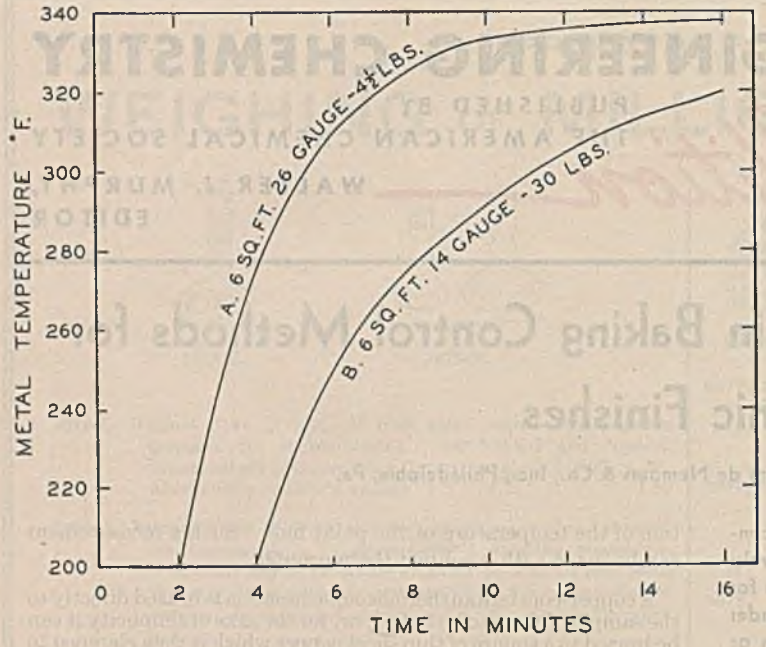

Figure 2. Time-Temperature Relationship

poor durability. As a matter of fact, the 30 pounds of heavy sheet must be baked about 16 minutes, or nearly twice as long, to obtain standard properties. The paint film on a 30-pound metal casting, of small surface area, is still tacky after the full 16 minutes in this oven. It must be baked approximately 40 minutes or nearly four times as long as the 26-gage metal to obtain usable film properties.

It is obvious that ware of varying thicknesses cannot be baked at the same high-temperature short-time schedule without encountering underbaking of the heavy pieces or overbaking with consequent poor color and brittleness of the light-weight pieces. This has been demonstrated in connection with numerous industrial applications where the operators have established the correct short baking schedule for sheet metal ware, only to find that heavy gear housings or other cast parts baked at the same time were so badly underbaked that gasoline used to remove grease after the subsequent assembling operation would wash off the finish.

There are also factors other than the mass of the metal which affect the baking speed of the finish—for instance, dark-colored bonderited metal sheets heat up much more rapidly than bright steel because of the heat reflectance from the backs of the latter. Enamels of varying colors have different heating rates for the same reasons. Various ovens heat a charge of ware at varying rates because of their varying heat capacities and rates of air circulation. In box-type ovens, the length of time during which the door is open for charging the oven has a very noticeable effect. These variables affect baking operations in all temperature ranges, but have a much greater effect on high-temperature bakes.

DETERMINATION OF BAKING VALUES

A method for determining the progress of an individual baking operation which will take into account the acceleration or deceleration of the bake caused by these various external influences is badly needed.

Since the curing or heat polymerization of an organic enamel film is essentially a chemical reaction, it should be possible to determine the relationship between temperature and velocity for this reaction, and by means of metal or paint film temperature determinations to calculate the progress of the polymerization reaction and determine the time at which it is completed.

The total baking effect on paint film is the summation of the individual baking effects obtained during short increments of time, such as one minute, the temperature during these increments varying from room temperature up to a maximum approaching or equaling the air temperature of the oven. It has been demonstrated that for the particular urea-formaldehyde type of industrial enamel with which this work was conducted, the reaction velocity at temperatures below 240° F. is so low that a minute of time below this temperature has a negligible baking effect, although the threshold temperature for other types of finishes might obviously be higher or lower. In Figure 3, the total bake on the ware is the sum of the baking effect represented by *a* and that represented by *b*, *c*, *d*, etc., each increment of time being, for the purpose of illustration, one minute.

The problem is now to determine the baking value of these various increments or, in other words, to determine the relationship between the reaction velocity and temperature for the particular product under consideration. Assuming that the reaction velocity *vs.* temperature curve for the polymerization reaction, like that for other chemical reactions, is logarithmic in nature, it should be possible to determine the reaction velocity at two or three relatively low temperatures where the baking time is long in comparison with the time required to bring the ware up to the temperature of the oven, and to extrapolate with reasonable accuracy to higher temperatures. At these low temperatures only slight errors are introduced by the necessity of estimating the effective starting time of the baking operation at the temperature under investigation. At the higher temperatures, the reaction may be completed before the test film reaches the temperature being studied.

Paint films have been applied to very thin sheet metal, which comes up to the oven temperature in a relatively short

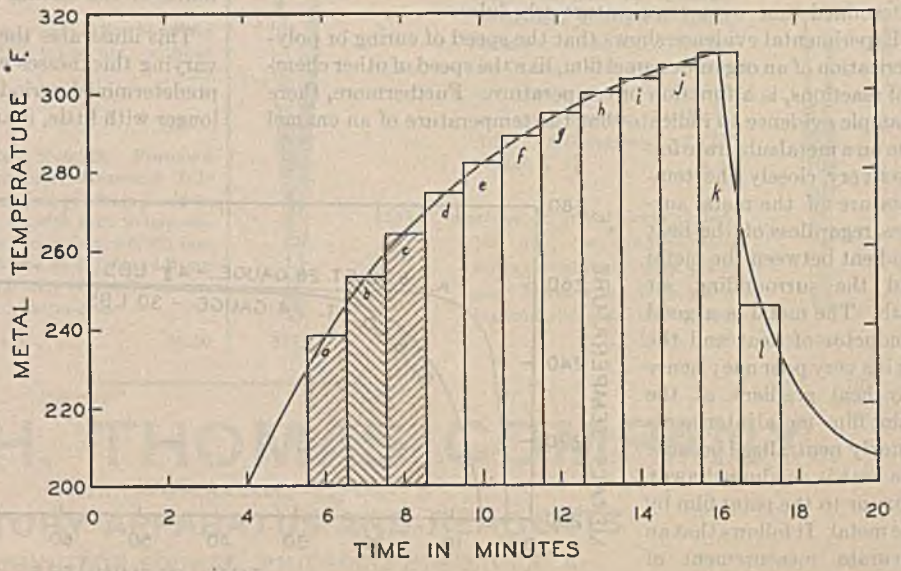

Figure 3

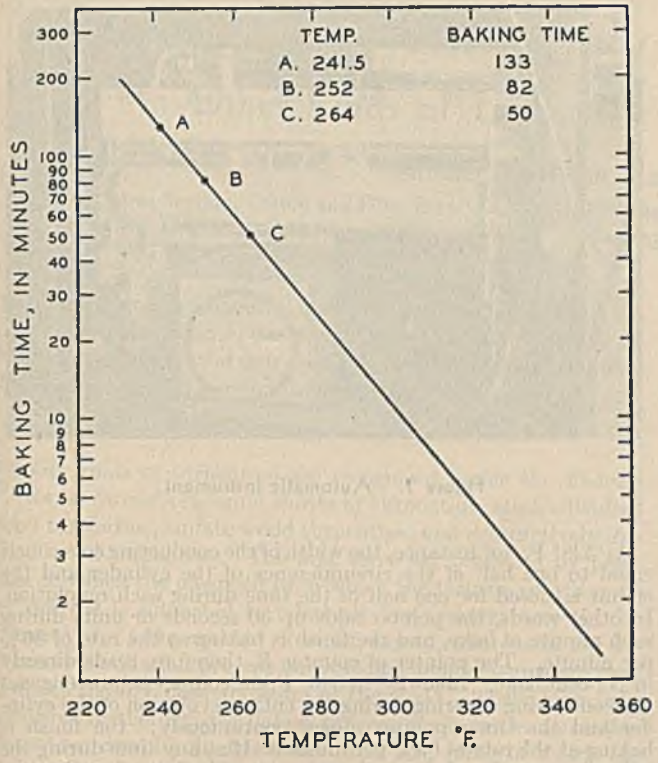

Figure 4. Time-Temperature Curve

time, and baked at 241.5°, 252°, and 264° F. until the arbitrarily selected standard film properties were obtained. By examining the films for these properties, the correct baking times for these temperatures have been determined to be, respectively, 133, 82, and 50 minutes. In Figure 4 these values were plotted on a logarithmic scale against temperature. As predicted, they lie roughly in a straight line, the error being well within the limit of error in the method of determining the reaction end point. The extension of this line represents the extrapolation of these points to higher temperatures.

For any given temperature the reciprocal of the baking time, or time in minutes required to give a complete bake, multiplied by 100, gives the per cent of bake acquired in one minute at that temperature. Figure 5 represents per cent bake per minute *vs.* temperature as converted from the baking time *vs.* temperature curve in Figure 4. It is plotted on a linear scale.

The curve was tested by using it as a guide, as described below, in making bakes at various temperatures from 240° up to 340° F. At all temperatures comparable bakes were obtained as judged by color, gloss, and other properties regardless of the oven loading, thickness of the metal, etc. The original assumptions concerning the relationship between baking speed and temperature, and the logarithmic shape of the reaction velocity *vs.* temperature curve are shown to be correct. Occasionally, when curves of this type are being calculated the expected errors of extrapolation are encountered, resulting in consistent over- or underbakes in the high-temperature range. In these cases the slope of the logarithmic curve must be re-established by trial and error methods.

The curve in Figure 5, then, provides a means for integrating the time-temperature curve for a baking operation or, in other words, for summing up the various baking values for the 1-minute increments as the bake proceeds. The metal temperature is determined every minute, converted by means of Figure 5 to per cent bake per minute, and added to the previous value. When a total of 100% is reached the ware is removed from the oven.

An examination of Figure 5 reveals many of the reasons for the difficulties previously encountered in controlling high-temperature, short-time baking operations. In the range of 340° (metal temperature) the paint film is baking at the rate of 50% per minute. In other words, a 1-minute variation in the time at which the ware is removed from the oven gives a 50% error in effective bake. In this temperature range a slump of 10° for only 1 minute, due to power failure, opening of the oven doors, etc., will result in a 15 to 20% decrease in effective bake. Because of the number of variables which affect baking operations, it is common to find variations of as much as 20° to 30° in metal temperature, during the heating up period, from one bake to the next, seemingly conducted under the same conditions. Figure 5

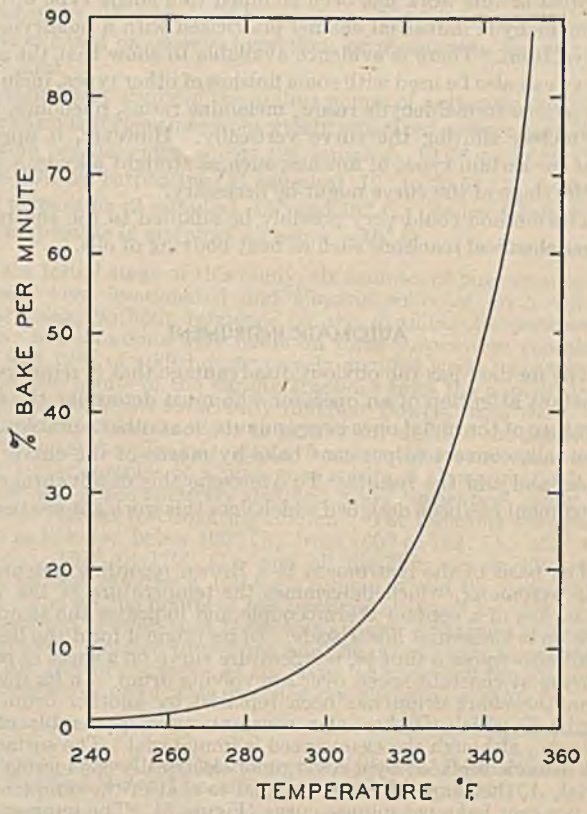

Figure 5. Integration of Time-Temperature Curve

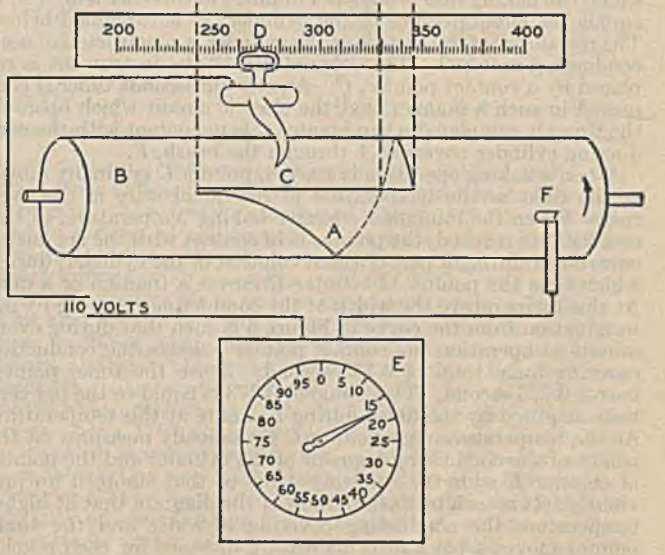

Figure 6. Diagram of Instrument

demonstrates the serious error in effective bake which will result from these variations. Attempts to control bakes by determining air temperature result in large errors because the metal temperature lags considerably behind the air temperature and experience has shown that the magnitude of this lag is entirely unpredictable, especially when oven charges of various weights per unit area are being handled.

The method of controlling baking operations as described has proved to be foolproof and has resulted in consistently equivalent film properties on various types of ware from thin sheet metal to heavy castings baked under widely varying conditions. However, precautions must still be taken against baking pieces of varying weights per unit area or varying colors during the same operation.

Most of this work has been confined to a single type of urea-formaldehyde industrial enamel plasticized with a nondrying oil alkyd resin. There is evidence available to show that the same curve can also be used with some finishes of other types, including other urea-formaldehyde resins, melamine resins, phenolics, etc., by merely shifting the curve vertically. However, it appears that for certain types of finishes, such as straight alkyds, a shift in the slope of the curve might be necessary.

The method could very possibly be adapted to the control of other chemical reactions such as heat bodying of oils.

AUTOMATIC INSTRUMENT

The method has the obvious disadvantage that it requires the constant attention of an operator who must determine the temperature of the metal once every minute or at other suitable short intervals, convert to per cent bake by means of the curve or a table, and add the results. To overcome this disadvantage, an instrument has been designed which does this work automatically.

The basis of the instrument is a Brown recording potentiometer pyrometer, which determines the temperature of the ware by means of a contact thermocouple and indicates the temperature on a horizontal linear scale. In its original form the instrument also traces a time *vs.* temperature curve on a sheet of paper moving at constant speed over a revolving drum. In its revised form the chart drum has been replaced by another drum (B, Figure 6) which revolves at a constant speed, preferably about 3 r.p.m., although the exact speed is immaterial. The surface of the drum is replaced by a covering of electrically conducting material, *A*, the shape of which is equal to that of the temperature *vs.* per cent bake per minute curve (Figure 5). The temperature axis of the curve is drawn to the same scale as the horizontal temperature scale of the potentiometer. The per cent bake per minute axis is drawn to such a scale that, at that temperature at which the baking rate is 60% per minute (in this case 345° F.), it equals the circumference of the cylinder, as is explained below. The remainder of the surface of the cylinder is constructed of nonconducting material. The original pen of the instrument is replaced by a contact pointer, *C*. An electric seconds timer is connected in such a manner that the electric circuit which operates the timer is completed when pointer *C* is in contact with the conducting cylinder covering *A* through the brush, *F*.

When a baking operation is started, pointer *C* gradually moves to the right as the temperature of the metal ware in the oven rises. When the minimum effective baking temperature, in this case 240°, is reached, the pointer is in contact with the conductor covering *A* during a part of the revolution of the cylinder, during which time the pointer of counter *E* moves a fraction of a unit. At this temperature the width of the conducting covering by determination from the curve in Figure 5 is such that during every minute of operation the contact pointer engages the conducting covering for a total of 0.773 second; hence the timer pointer moves 0.773 second. This value of 0.773 is equal to the per cent bake acquired by the finish during 1 minute at this temperature. As the temperature rises pointer *C* periodically measures off the height of the conducting segment of the cylinder and the pointer of counter *E* adds the resulting value to that summed up previously. It is seen by examination of the diagram that at higher temperature the conducting covering is wider and the timer pointer moves a constantly increasing distance for each revolution of the cylinder.

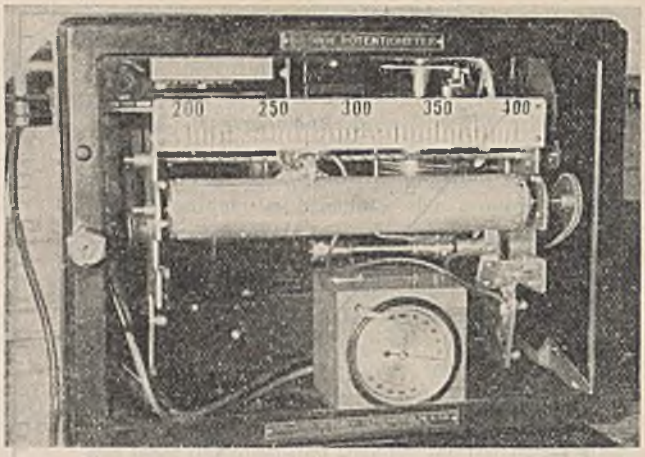

Figure 7. Automatic Instrument

At 328° F., for instance, the width of the conducting covering is equal to one half of the circumference of the cylinder and the circuit is closed for one half of the time during each revolution. In other words, the pointer adds up 30 seconds or units during each minute of bake, and the finish is baking at the rate of 30% per minute. The pointer of counter *E*, therefore, reads directly in per cent bake. Likewise, at 345° F. the contact pointer engages the conducting covering during the entire revolution of the cylinder and the timer pointer moves continuously; the finish is baking at the rate of 60% per minute. If at any time during the bake the oven door is opened or the power is shut off so that the temperature of the metal drops off, the instrument will follow this dip in temperature, since the number of units which the counter pointer moves per unit of time will also decrease.

As the bake proceeds, it is only necessary to glance periodically at the pointer. As it approaches 100% the ware is removed from the oven.

In the case of another type of finish with a faster baking speed but whose per cent bake per minute *vs.* temperature curve has the same slope, it is necessary only to apply a predetermined factor, baking, for instance, to 75% instead of 100%. If the curve has a different slope, it is necessary to construct an interchangeable cylinder for the instrument. Cylinders can also be constructed to cover a wider range of baking temperatures, in which case it is necessary to make suitable alterations in the scale of the electric timer to agree with the revised ordinate of the conducting curve on the cylinder.

A photograph of the instrument is shown in Figure 7.

Volume Correction Factors for C_4 Hydrocarbon Mixtures

National Bureau of Standards Letter Circular LC-757, compiled by C. S. Cragoe, presents in 23 pages volume correction factors for C_4 hydrocarbon mixtures compiled at the request of the Rubber Reserve Co. to supplement tables on pure hydrocarbons issued November 23, 1943, to supply standard tables especially applicable to C_4 mixtures covering a wide range of composition, to facilitate accurate determinations of quantities bought and sold in commercial transactions, and to supersede other less accurate tables. One of its major objectives is standardization of methods of correcting volumes observed at various temperatures to the conventional standard units (gallons at 60° F.) in commercial transactions involving C_4 hydrocarbon mixtures, particularly those used in the manufacture of aviation gasoline and butadiene. Copies are available on request from the National Bureau of Standards, Washington 25, D. C.

Detection of Destructively Distilled Wood Turpentine

In Other Kinds of Turpentine by Means of the Aniline Point

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The presence of destructively distilled wood turpentine in gum spirits, and perhaps also in steam-distilled wood turpentine, may be detected by aniline point tests on the high-boiling fraction distilling above 170° C., described in this paper.

FOUR kinds of turpentine are recognized under the Federal Naval Stores Act—gum spirits of turpentine, steam-distilled wood turpentine, sulfate wood turpentine, and destructively distilled wood turpentine. The first three are produced by processes in which the oleoresin from which the terpene constituents are derived is subjected to relatively low temperatures (in the presence of a large amount of water vapor), whereas in the destructive distillation process much higher temperatures are used to effect the dry decomposition of the wood. These high temperatures in the dry distillation process result in the formation of various complex hydrocarbons and oils, some of which are closely related to the aromatic or benzene hydrocarbons, and some are perhaps of unknown identity.

Destructively distilled wood turpentine usually sells at a price below the established market price for gum spirits of turpentine or steam-distilled wood turpentine. The price differential has at times induced unscrupulous dealers to adulterate gum spirits and steam-distilled wood turpentine by adding small quantities of the destructively distilled wood turpentine. The sale of mixtures of this kind in interstate commerce is injurious to commerce in naval stores and prejudicial to the sale of pure turpentine, and is therefore prohibited by the Federal Naval Stores Act. The wholesale price of sulfate wood turpentine is also usually below that of the other two kinds; consequently, its adulteration would hardly be economically feasible.

This type of adulteration can usually be detected, especially by a person experienced in the testing of turpentine, because of the characteristic odor of the adulterant. However, to provide legally acceptable evidence, the analyst needs some method of evaluation based on scientific fact or recordable data—entirely independent of the personal element based on a sensory observation—on which to support his findings.

As indicated by the standard specifications under which the several kinds of turpentine are produced and sold, destructively distilled wood turpentine contains appreciable quantities of constituents distilling in the range from 170° to 180° C. (*1*, *4*), whereas in gum spirits and steam-distilled turpentine (*1*, *3*) which consist chiefly of α- and β-pinene, these higher boiling constituents are present in only relatively small quantity.

Another difference between destructively distilled and other types of turpentine owing to the difference in composition, is the greater so-called "solvent power" of the former. The two most commonly used methods for evaluating the solvency of paint thinners are the kauri-butanol (*5*) and the aniline point tests (*6*). The kauri-butanol test is subject to wide variation due to difficulty in temperature control, and each new solution of kauri gum must be standardized to establish reference points. The aniline point test, on the other hand, is relatively simple, is the only commonly used solvency test in which close temperature control is not a factor, and is widely used to evaluate paint thinners and diluents. (The aniline point of a diluent or solvent is the minimum equilibrium solution temperature for equal volumes of aniline and the solvent.) It was, therefore, considered by the authors that these two differences in properties might serve as a means of proving the presence of destructively distilled wood turpentine in other kinds of turpentine. No reliable method of detection based on chemical reactions or phenomena has as yet been found.

A preliminary study of the aniline points of authentic samples of the various turpentines from widely separated sources gave the following results: gum spirits (24 samples), 12.2° to 14.5° C.; steam-distilled turpentine (11 samples), 19° to 25.5° C.; sulfate wood turpentine (2 samples), 15° and 18° C.; destructively distilled turpentine (4 samples), all below −10° C.

In the initial stage of this study, six samples of pure gum turpentines were fractionated and aliquots collected on a volumetric basis, without reference to the distilling temperature. Similar fractionations were made on these turpentines containing 5 and 10% of added destructively distilled wood turpentine. The aniline points of the various fractions from the adulterated turpentine were not sufficiently different (lower) from those of the pure turpentine fractions to permit any definite conclusions.

After several preliminary tests, fractionations were made on single 1-liter samples of a pure gum spirits, a steam-distilled, a sulfate, and a destructively distilled wood turpentine, using a 250-mm. Vigreux fractionating column. The fractions were collected as follows: below 160° C.; from 160° to 163° C.; 163° to 167° C.; 167° to 170° C.; and all distilling above 170° C. (With smaller samples a 150-mm. column would be more suitable.) The aniline points of the fractions obtained by this type of separation showed that there was enough difference between destructively distilled turpentine and the other kinds to suggest that this test might serve as the basis for a method for positive identification or proof of its presence in a suspected mixture.

Table I. Mixed Aniline Points of Fractions of Destructively Distilled Wood Turpentine, Collected Above 170° C.

Mineral Spirits (60° C., A.P.)	Sample Number			
	1	2	3	4
%	° C.	° C.	° C.	° C.
0	<−10.0	<−10.0	<−10.0	<−10.0
10	− 6.0	−10.5	− 3.0	− 7.0
20	2.0	1.5	9.5	3.0
30	11.5	9.5	16.0	12.5
40	18.5	18.5	23.0	20.0
50	25.5	26.5	30.0	26.5
Yield of distillate over 170° C., %	18.4	16.0	50.3	25.2

Four authentic samples of destructively distilled wood turpentine from different producers were next fractionated through the column. The fractions collected above 170° C. were subjected to a series of aniline point tests, both on the fraction as collected and after mixing with varying proportions of a standard mineral spirits having an aniline point of 60° C. The similarity of the aniline points for these four samples is shown in Table I.

The next step in the study was the comparison of the high-boiling fractions, when recovered by distillation at atmospheric pressure, and when obtained at reduced pressure. Sample 1 was considered characteristic and was selected. It was first fractionated at atmospheric pressure throughout the distillation, the fraction above 170° C. being reserved. A similar distillation was made at atmospheric pressure up to 170° C., after which the higher fraction was collected at reduced pressure (40 mm.).

Five per cent by volume of this same turpentine was added to a quantity of one of the pure gum turpentines, and two similar fractionations were made on this adulterated sample. The four high-boiling fractions were subjected to a series of mixed aniline point tests (Table II). The results showed that even with as

little as 5% of destructively distilled wood turpentine in gum spirits a portion remains undistilled at 170° C. under atmospheric pressure; also that further distillation of this residue, either at atmospheric or preferably at reduced pressure, yields a fraction which has mixed aniline points that are in good agreement with those obtained on the similar fraction from the straight destructively distilled wood turpentine.

For the last series of tests, one authentic gum spirits and three authentic destructively distilled wood turpentines were selected. All four samples were first subjected to fractional distillation at atmospheric pressure. A series of mixed samples was prepared, each to contain destructively distilled turpentine in the gum turpentine, in the ratios of 1 to 19 (5%) and 1 to 9 (10%), and fractionated in the same manner. The straight aniline points of these fractions were determined (Table III).

Table II. Mixed Aniline Points

(Fractions of D.D. wood turpentine collected above 170° C. and of gum turpentine containing 5% of same D.D. wood turpentine)

Mineral Spirits (60° C., A.P.)	D.D. Wood Turpentine Straight distillation	D.D. Wood Turpentine Vacuum distillation[a]	95% Gum–5% D.D. Straight distillation	95% Gum–5% D.D. Vacuum distillation[a]
%	° C.	° C.	° C.	° C.
0	<−10.0	<−10.0	<−10.0	<−10.0
20	0.5	− 3.0	2.5	− 5.0
40	18.5	16.5	20.5	14.0
60	34.5	33.5	35.5	32.0
80	48.0	48.0	48.5	47.0
Distillate, %	28.0	30.8	2.1	5.0

[a] Material subjected to vacuum distillation was that remaining after atmospheric distilling temperature reached 170° C.

From the data obtained it is concluded that any opinion as to the presence of destructively distilled wood turpentine in gum spirits of turpentine, based on an olfactory detection of the characteristic pungent odor of the former, may be substantiated by aniline point tests on the higher boiling fractions. The odor of the adulterant, when present in only small quantity, was more easily recognized in the higher fractions.

Since the straight aniline points of the samples of gum spirits of turpentine were closer to those of the destructively distilled wood turpentines than was the case with the steam-distilled wood turpentines initially tested, it was felt that any conclusions or procedure based on a depression of aniline point of fractions from a gum spirits would hold for most steam-distilled wood turpentines. No comparable series of tests was therefore made with this latter class.

Steam-distilled wood turpentine, which is produced by a rather complicated refining process from the original distillate as it comes from the wood, may be poorly refined and contain small quantities of dipentene or pine oil which might also serve to depress the aniline points of the higher fractions. However, these substances are usually removed by the manufacturer to the greatest extent possible, since they have become more valuable than the turpentine. They must be removed if the turpentine is to meet the standard specifications for this class, which are identical with the specifications for gum spirits. The chances of drawing false conclusions from the aniline point data obtained by means of the procedure herein described are believed to be remote enough to warrant the use of the method also on samples of steam-distilled wood turpentine suspected of this type of adulteration, especially when the presence of the destructively distilled wood turpentine is suggested, even though faintly, by the odor.

The small quantities of material on which the aniline point tests had to be run, and the low temperatures obtained, made it necessary to develop a test assembly different from the apparatus used in the standard A.S.T.M. aniline point determination (*2*). The apparatus is shown in Figure 1.

Test tube *B* is supported by a heavy cardboard cover over *C*, which at the same time serves to insulate the surface of the liquid therein from the surrounding warmer atmosphere.

For most purposes, the following procedure should give satisfactory and reliable results. Fractionate from 250 ml. to 1 liter of the sample through an efficient distilling column, at atmospheric pressure, carrying the temperature of the distilling vapor up to 170° C. The distillate up to this point is not used. Continue the fractionation, if possible, under slight vacuum, until the distillation stops or decomposition begins. Dry this fraction by shaking with anhydrous sodium carbonate, and filtering, preferably through a fritted glass-bottom crucible of medium porosity. Pipet 3 ml. of the dried sample into test tube *A* and add 3 ml. of pure fresh aniline. (Only pure, fresh aniline will serve the purpose. Old aniline must be purified by redistilling after treatment with anhydrous sodium carbonate, rejecting the first and last 10% of distillate. As aniline is toxic, even through the skin; it should be handled with caution and should never be directly pipetted by placing the end of the pipet itself in the mouth.)

Fill baths *B* and *C* with prechilled alcohol or acetone, the temperature of which is gradually reduced by the addition, at intervals, of small pieces of dry ice. However, if this is not available, it is possible to obtain a temperature of −21° C. by using a mixture of equal parts of 66% sulfuric acid, precooled to 0° C., and finely crushed ice. Immerse the tube in the chilling or low-temperature bath, *B*, until the solution in *A* clouds. Remove the tube and agitate the contents with the copper wire in a shuttle-like manner during the observation of the aniline point. Read the temperature at the instant the solution clears. Repeat the operation until duplicate operations or readings agree within ±0.2° C.

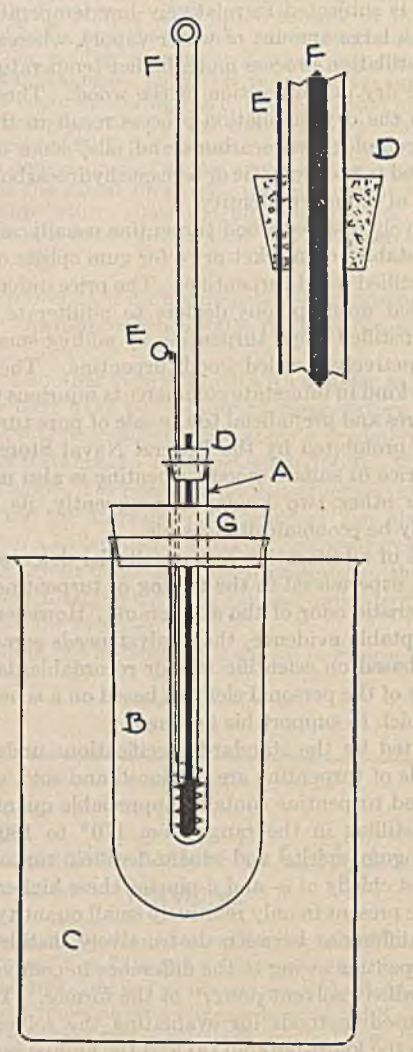

Figure 1. Assembly for Determining Aniline Points

A. 15-ml. test tube, with lip, 12.5 × 1.25 cm. (5 × 0.5 inches)
B. 200-ml. test tube, with lip 12.5 × 5 cm. (5 × 2 inches)
C. 100-ml. beaker
D. Cork stopper, to exclude moisture
E. Copper wire for agitation, coiled at base
F. Low aniline point thermometer −38 to +42° C., A.S.T.M., E-1 (33C-41-T)
G. Cork stopper

CONCLUSIONS

Recordable data to prove the presence of destructively distilled wood turpentine in gum spirits, and perhaps in steam-distilled wood turpentine, may be had by subjecting the high-boiling fraction distilling above 170° C. to an aniline point determination. The presence of such adulteration, particularly if also indicated by odor, would be verified: (1) if the straight aniline point of the whole sample is lower than +12° C.

(2) if a distillation fraction, using a column, is obtained which distills above 170° C.

(3) if the straight aniline point of this fraction is lower than −10° C.

(4) if the mixed aniline point of such fraction, using a 50-50 mixture of the fraction and a standard 60° C. A.P. mineral spirits, is lower than +30° C.

Table III. Aniline Points of Fractional Distillates of Gum Spirits and Destructively Distilled Wood Turpentine and Mixtures Thereof

	Original Sample				Mixed Sample Gum with					
	Gum	D.D. No. 1	D.D. No. 2	D.D. No. 3	5% No. 1	10% No. 1	5% No. 2	10% No. 2	5% No. 3	10% No. 3
	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.	° C.
Whole Sample	12.2	−20.4	−20.0	−20.8	10.5	9.1	10.4	9.0	10.2	8.8
Fraction distilling										
Below 160° C.	17.6	−24.2	−23.0	..	15.7	14.8	16.1	14.3	17.3	16.3
160–163° C.	13.4	−22.0	−19.0	..	11.5	11.9	12.4	11.4	13.1	12.0
163–167° C.	3.8	−13.0	−15.0	− 9.0	2.7	3.1	3.5	2.1	5.1	7.0
167–170° C.	−10.6	−15.0	−17.0	− 9.0	− 9.0	− 8.0	−10.4	− 9.0	− 5.0	− 3.5
Above 170° C.	..	−22.0	−21.4	−18.6	<−30.0	−16.8	−22.0	<−30.0	−15.0	−14.4

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Physical Constants of Methyl Esters of Commonly Occurring Fatty Acids

VAPOR PRESSURE

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Methyl esters of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and linolic acids were obtained in a pure state by repeated fractional distillation. Vapor pressure curves and decomposition pressures and temperatures have been determined for each of the methyl esters by the method described by Ramsay and Young. With the exception of the C_{18} series, it has been shown that an ester fraction can be identified and its purity ascertained by means of its vapor pressure curve. With the aid of the decomposition data, it is possible to eliminate excess decomposition by controlling the pressure and hence the boiling temperature of fractional distillation.

FRACTIONAL distillation is recognized as an excellent method for separating and purifying the methyl esters of the naturally occurring fatty acids. The fractions obtained from such a distillation may be divided into two classes: the pure ester fractions, and the mixed fractions containing two or more esters.

Two types of analyses are possible for the identification of such fractions. The best and most commonly used are chemical analyses which involve the determination of such values as the iodine number, neutral equivalent, and thiocyanogen number. These procedures are difficult and time-consuming, and are impractical when the fractions are very small. Accordingly, it seemed advisable to investigate the second possibility, that of substituting physical measurements for the usual chemical methods. Consequently, the problem resolved itself into a search for suitable physical constants which would yield information as to the purity and quantity of any ester in a given fraction.

Vapor pressure was the first physical constant investigated. By means of this determination the purity of a substance may be ascertained in a very short time and with a considerable degree of accuracy. By choosing a dynamic method, it is also possible to find the boiling point of the substance in question at any given pressure. This last fact in itself is of great importance in the separation and purification of materials by fractional distillation. It is also possible to learn from a vapor pressure determination the temperature and pressure at which decomposition occurs. This information can be utilized to great advantage in the fractional distillation of organic compounds.

PREPARATION OF THE METHYL ESTERS

The methyl esters of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, and oleic acids were prepared and purified by repeated fractional distillation through a 16-plate, electrically heated fractionating column, of the type described by Whitmore and Lux (*2*), fitted with a total reflux, partial take-off distilling head. The purity of each ester was determined by the usual chemical analyses, consisting of neutral equivalent, iodine number, and thiocyanogen number. The methyl ester of linolic acid was purified first, by repeated crystallization of the tetrabromo derivative, and then, after regeneration, by repeated fractional distillations. These pure esters were used for the entire study of the physical constants.

DETERMINATION OF VAPOR PRESSURE

The vapor pressure curve for each methyl ester was determined by the dynamic method described by Ramsay and Young (*1*); which was found to give very accurate results for all esters, including those which are normally solid at room temperature. This method was chosen because it requires a very small amount of material (approximately 1 ml.) which can be recovered, providing no decomposition takes place during the determination.

In Figures 1 and 2 are shown the vapor pressure curves for the pure methyl esters of the more commonly occurring fatty acids. The straight-line curves, drawn on semilog paper, were obtained by plotting the reciprocal of the absolute temperature × 1000 against the log of the pressure in millimeters of mercury. The curves, thus constructed, were extrapolated to 760-mm. pressure,

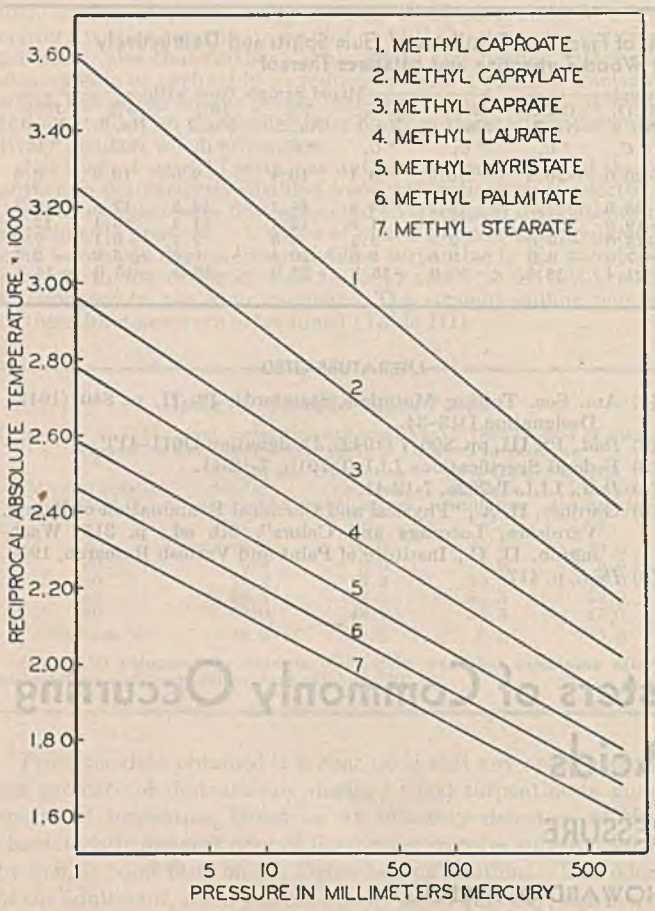

Figure 1. Vapor Pressure Curves of Methyl Esters of More Commonly Occurring Fatty Acids

since in the majority of the cases decomposition occurs far below that pressure. Table I gives the approximate decomposition pressures and temperatures for each of the methyl esters studied. Table II shows the boiling points for each of the esters at several different pressures.

CONCLUSIONS

From the data collected, several significant conclusions may be drawn.

With the exception of the C_{18} series, whose vapor pressure curves as shown by Figure 2 are practically identical, it is possible to identify a pure ester fraction by means of its vapor pressure curve. With the above information available, it is an easy matter to find the boiling point of an unknown ester at several different pressures, and then by superimposing the curve thus constructed on one of the above standard curves, to identify the ester. Such identifications can be accomplished in a very short time and with a considerable degree of accuracy. The purity of a known ester may be determined in the same way. The ester is pure if the constructed curve can be superimposed on the standard vapor pressure curve of the ester in question. Any slight deviation from purity will be recognized immediately, inasmuch as the two curves will not coincide.

Table I. Decomposition Pressures and Temperatures of Methyl Esters of Fatty Acids

Ester	Pressure, Mm. Hg	Temperature, ° C.
Methyl caproate	>760	>150
Methyl caprylate	>760	>193
Methyl caprate	>760	>224
Methyl laurate	160	204
Methyl myristate	60	205
Methyl palmitate	25	151
Methyl stearate	18	221
Methyl oleate	16	217
Methyl linolate	11	208

Table II. Boiling Points of Methyl Esters at Various Pressures

Ester	Pressure in Millimeters of Hg						
	2	4	6	8	10	20	40
	° C.	° C.	° C.	° C.	° C.	° C.	° C.
Methyl caproate	15	26	33	38	42	55	70
Methyl caprylate	45	58	65	71	76	89	106
Methyl caprate	77	89	97	103	108	123	139
Methyl laurate	100	113	121	128	134	149	166
Methyl myristate	127	141	150	157	162	177	197
Methyl palmitate	148	162	172	177	184	202	[a]
Methyl stearate	166	181	191	199	204	[a]	[a]
Methyl oleate	166.2	182	192	199.5	205.3	[a]	[a]
Methyl linolate	166.5	182.4	193	199.9	206	[a]	[a]

[a] Decomposes.

It is possible to adjust the pressure and hence the boiling temperature of a fractional distillation to optimum or nearly optimum conditions by making use of the recorded decomposition pressures and temperatures. The authors believe that during a fractional distillation there is a tendency toward unnecessary superheating of the material to be distilled. This tendency can be eliminated through the use of decomposition data on the various esters.

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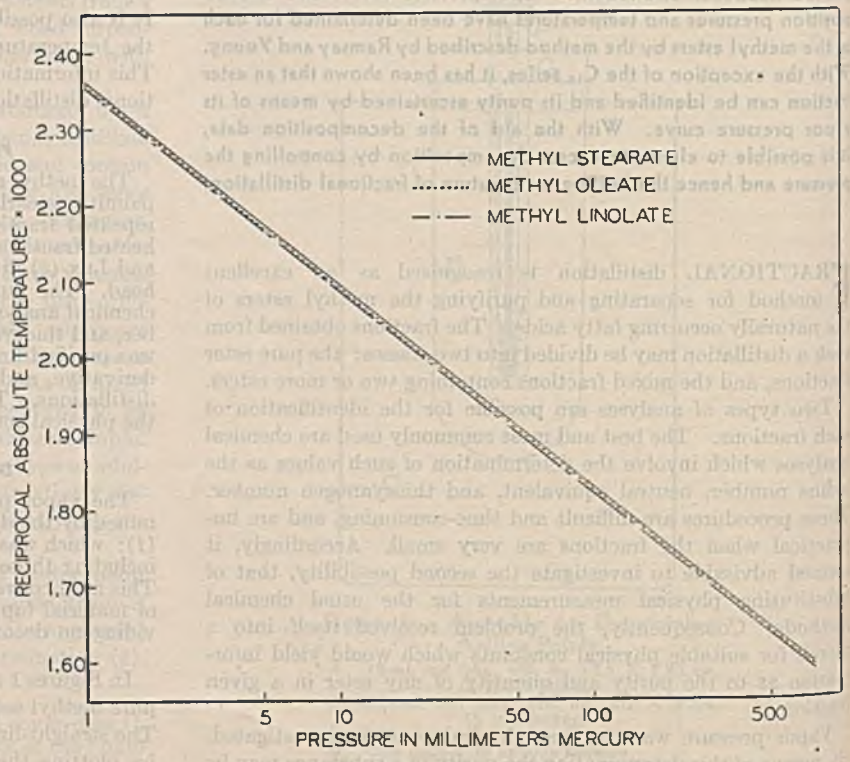

Figure 2. Vapor Pressure Curves of Methyl Esters of More Commonly Occurring Fatty Acids

Spectrographic Determination of Small Amounts of Tungsten in Steel

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Accurate determinations of 0.01 to 0.25% of tungsten in steels were made by a combined spectrochemical method. The tungsten was separated by a variation of Knowles' method and then determined spectrographically. A modified chemical procedure for separating all the columbium from tungsten was developed in order to determine the tungsten spectrographically. A high degree of reproducibility was obtained by sparking carbon electrodes impregnated with the tungsten solution.

THE determination of small amounts of tungsten (0.01 to 0.25%) in steel has not been accomplished with satisfactory accuracy up to the present time. In the colorimetric determination of tungsten interference is caused by trace amounts of molybdenum (*17–20*). To date no successful spectrographic procedure has been reported which allows the determination of tungsten in the above-mentioned range, using solid steel specimens directly or a simple solution procedure (*3, 7, 10, 11, 14, 15*). Thus a project of combining both chemical and spectrographic methods was undertaken in determining residual tungsten in steel.

The tungsten is first separated chemically by a modification of Knowles' method (*8, 18, 19*) and all but a trace of molybdenum is removed by volatilization. The spectrographic procedure is then employed, using an alkaline solution of the tungstic oxide, with an aluminum salt added as an internal standard. A spark solution method (*16*) is employed which has a high degree of reproducibility, unobtainable in this case by the usual arc method (*2, 10, 12, 13, 14*). The use of a medium spectrograph with its high optical efficiency and sufficient resolving power was found to be satisfactory. The tungsten content can then be determined accurately by densitometric measurements.

CHEMICAL PROCEDURE

Dissolve a 2-gram sample in a covered 250-ml. beaker with 10 ml. of hydrochloric acid (sp. gr. 1.19) and 30 ml. of perchloric acid (70%). Heat over a low flame until the sample is completely dissolved, rinse, remove the cover glass, then add 1 to 2 ml. of hydrofluoric acid (48%). Increase the heat and evaporate to fumes of perchloric acid. When the chromium begins to oxidize, denoting the decomposition of the carbides, remove the beaker from the heat and allow it to cool until the salts begin to crystallize. Wash down the sides of the beaker with about 20 ml. of distilled water and swirl the salts into solution. Add 20 ml. of saturated sulfurous acid and heat until the sulfur dioxide is completely driven off. Cool to 10° C. in an ice bath, then add 3 ml. of ammonium molybdate solution (0.0054 gram of molybdenum per ml.) and some paper pulp and mix well. Add slowly 15 ml. of a 2% alcoholic solution of α-benzoin oxime while stirring and a sufficient quantity of saturated bromine water to tint the solution orange.

Add 15 ml. more of the α-benzoin oxime solution and repeat the foregoing procedure. Allow to stand 10 minutes with occasional stirring, adding more bromine water if necessary. Filter through a No. 40 11.0-cm. Whatman paper containing paper pulp without allowing the precipitate to run dry. Break up the precipitate with α-benzoin oxime wash solution (10 ml. of sulfuric acid sp. gr. 1.84, 965 ml. of distilled water, cool to 10° C., and add 25 ml. of alcoholic α-benzoin oxime solution, 2%). Rinse and swab the beaker, and wash the precipitate with 150 ml. of the wash solution, breaking up the precipitate. Char and ignite in a platinum crucible at 734° C. for 2 hours or until there is no further sign of molybdenum volatilizing. Add to the residue in the platinum crucible 5.0 ml. of a stock solution containing 490 ml. of sodium hydroxide (0.5 molar) and 10 ml. of aluminum sulfate octadecahydrate (10%). Stir with a glass rod to macerate any lumps in the residue. Warm on a steam bath for a few minutes, then filter through a dry No. 40 Whatman 9.0-cm. paper.

MODIFIED CHEMICAL PROCEDURE WHEN COLUMBIUM IS PRESENT. Dissolve a 2-gram sample in a covered 250-ml. beaker with 10 ml. of hydrochloric acid (sp. gr. 1.19), 5 ml. of phosphoric acid (85%), and 30 ml. of perchloric acid (70%). Heat over a low flame until the sample is completely dissolved. Continue as in chemical procedure.

SPECTROGRAPHIC PROCEDURE

The carbon electrodes used in the spectrographic procedure are prepared by heating graphitic rods 0.78 × 30 cm. ($^5/_{16}$ × 12 inches), in a muffle at 510° C. for 1 hour. After cooling, the rods are cut into 5-cm. (2-inch) lengths on an alundum cutting wheel (*16*). Before using, the ends of the electrodes are polished with a fine file. One drop of the solution, prepared by the above chemical procedure, is placed on each of two flat top carbon electrodes, which are sparked immediately after absorption of the solution. A Bausch & Lomb medium quartz spectrograph is used with an uncontrolled, condensed spark source, rated at 13,500 volts. The optical stand is placed 45 cm. (18 inches) from the slit. An exposure of 60 seconds with no condensing lens or pre-spark is recorded on an Eastman 33 plate 10 × 25 cm., (4 × 10 inches). The plate is developed for 4 minutes in D-19 at 18° C. and fixed for 15 minutes, followed by the usual process of washing and drying.

Figure 1. Spectra

1. 0.01% tungsten 2. 0.05% tungsten 3. 0.10% tungsten

The calibration of the plate is based on photometric measurement of lines having predetermined intensity values (*1*). The densities of the 2397.091 Å. tungsten line and the 2378.408 Å. aluminum reference line (*4*) are measured on a Leeds & Northrup recording microphotometer. The tungsten is then determined by the use of a working curve drawn by plotting $\Delta \log I \frac{W}{Al}$ against percentage tungsten. Standard tungsten solutions are prepared for determining the values of the curve.

PREPARATION OF STANDARDS

STANDARD TUNGSTEN SOLUTION (1 ml. = 0.001 gram of tungsten). One gram of pure tungsten metal is oxidized completely to yellow tungstic oxide in a muffle at 734° C. The tungstic oxide is then dissolved by warming in a solution containing 25 grams of sodium hydroxide in 100 ml. of distilled water. When solution is complete, it is transferred to a 1000-ml. volumetric flask and diluted to the mark.

A check of the standard solution is made by withdrawing 100 ml. of the solution and determining the tungsten by the cinchonine method (*6*).

SPECTROGRAPHIC STANDARDS

To a corrosion-resistant steel (18–8) containing no tungsten, increments of the standard tungsten solution were added to give a

Table I. Reproducibility and Accuracy of Determinations[a]

Sample No.	Tungsten Added %	Δ Log I	Maximum Deviation %
1	0.01	−56	
2		−51	
3		−54	0.002
4		−55	
5		−54	
1	0.03	−14	
2		−15	
3		−15	0.003
4		−18	
5		−18	
1	0.05	+04	
2		+02	
3		+06	0.006
4		+05	
5		+02	
1	0.07	+18	
2		+19	
3		+15	0.006
4		+16	
5		+17	
1	0.10	+32	
2		+32	
3		+32	0.003
4		+32	
5		+33	
1	0.12	+39	
2		+39	
3		+40	0.006
4		+39	
5		+41	

[a] Values for 0.13 to 0.25% tungsten determined from 1-gram samples by doubling results obtained.

range from 0.01 to 0.12% tungsten based on a 2-gram sample. For the range 0.13 to 0.25% tungsten, the same procedure was followed, based on a 1-gram sample. The tungsten separations were carried out according to the outlined chemical procedure. The spectrographic working curve was obtained from the values of these standards.

DISCUSSION

The three spectra in Figure 1 show the gradation of the tungsten line 2397.091 Å., and the position of the aluminum reference line 2378.408 Å. The results in Tables I and II show the reproducibility and accuracy obtained for tungsten standards

Table II. Accuracy of Determinations

Bureau of Standards Sample	Type	Tungsten Present[a] %	Tungsten Found %	Deviation from Actual %
73a	Alloy, high Cr, low Ni	0.09	0.085	0.005
			0.087	0.003
			0.085	0.005
			0.085	0.005
123a	Corrosion-resistant steel, containing columbium	0.11	0.115	0.005
			0.103	0.007
			0.110	0.000
			0.108	0.002

[a] From Bureau of Standards certificate of analysis.

Bureau of Standard samples 73a and columbium-bearing corrosion-resistant steel 123a. The spectrographic standard solutions, as described, were used in the construction of the working curve. Pure tungsten solutions were not used, since it was considered desirable to approximate the conditions of routine analysis. Investigation showed that a working curve, prepared by using pure tungsten solutions, differed from one constructed by the above procedure. Although tungsten is claimed to be completely precipitated by α-benzoin oxime (*9*), the authors feel that further investigation of the completeness of this precipitation and other possible causes of this difference is a separate problem and beyond the scope of this paper.

A modification of the chemical procedure must be employed in the analysis of columbium-bearing steels, since interference exists between tungsten and columbium under the discharge conditions employed and with the dispersion and resolution available with the authors' spectrograph. However, tungsten and columbium are not ordinarily easily separated (*5*), so that a modified chemical procedure was developed to permit such separation. The use of phosphoric acid in the modified chemical procedure causes a partial but proportional precipitation of tungsten in its separation from columbium. The working curve drawn up from this method (Figure 2, *B*), is therefore different from the curve (Figure 2, *A*) constructed from the original procedure.

This modification is applicable to all steel alloys, eliminating the use of two curves. However, it is advisable to avoid unnecessary loss of tungsten when columbium is not present. This

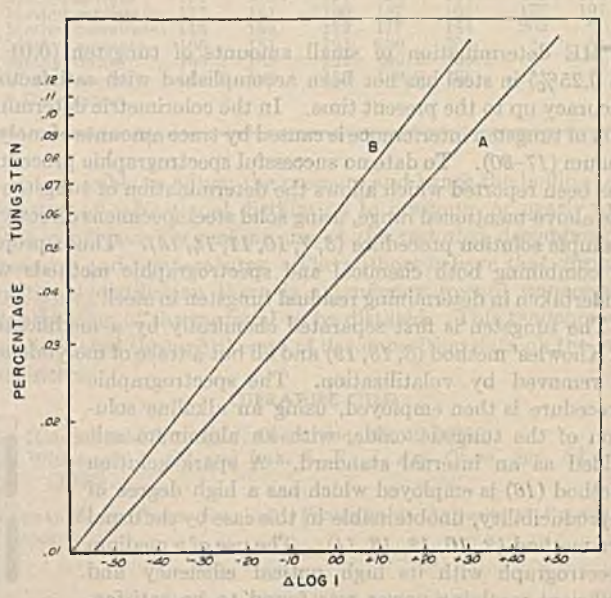

Figure 2. Working Curves
A. Corrosion-resistant steel, carbon tool steel
B. Columbium-bearing steel

is important when we consider that the amount of tungsten present in the solution to be analyzed is very small—i.e., 0.01% tungsten based on a 2-gram sample is equivalent to 0.0002 gram of tungsten. Furthermore, the number of columbium-bearing steel analyzed for small amounts of tungsten is negligible in proportion to the total number of samples received in this laboratory.

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This paper is not to be construed as an official method of the Navy Department.

Amino Acid Analysis of Some Common Vegetables

Method for Carbohydrate-Free Extraction of Nitrogen from Fresh Vegetables

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Fresh vegetables (0.5 to 1.0 kg.) were frozen, cut to suitable size, and immersed in acetone at room temperature for 48 hours, then submitted to a continuous acetone extraction for 24 hours. The two acetone fractions were combined and the nitrogenous products washed out by this treatment were set aside for analysis after the removal of acetone, lipids, and plant pigments. The vegetable residues were further extracted by 24-hour immersions in each of two 1.5 liters of hot 90% formic acid. These two fractions were combined and concentrated in vacuo to 1 liter, and the carbohydrates were precipitated by the addition of 2 liters of 95% ethanol and removed by filtration. The filtrates, which contain the bulk of the nitrogen, were distilled in vacuo to remove the alcohol and formic acid. The combined acetone-soluble and formic acid–soluble residues were found suitable for bioassay or on hydrolysis for amino acid analyses, and contained 90 to 95% of the total nitrogen of the fresh products.

THE current state of knowledge regarding the protein composition of vegetable foods has been recently assessed by Vickery (*21*): "What is needed is a statement of the amino acid composition of the total proteins of these vegetable products. What is to be found in the literature are more or less incomplete and seldom trustworthy tables of the composition of purified samples of the chief protein component." Because of the world-wide depletion of animal proteins and the consequent increased human consumption of fresh vegetable foods brought about by the present crisis, the need for securing this information has become most urgent. Consideration of these circumstances and the newer knowledge on the effect of deficiencies of certain amino acids in man and experimental animals (*1*, *2*, *3*, *5*) led the authors to initiate a program of study in 1942, with the purpose of obtaining data on the essential amino acid content of whole fresh vegetables.

The ultimate cause of the lack of suitable data on the amino acid composition of vegetables is the lack of a method for the complete and carbohydrate-free extraction of proteins from vegetable products. It would seem that all that is required is to submit samples of the vegetables directly to hydrolysis and to perform amino acid analyses on the hydrolyzates. The shortcomings of this direct approach became obvious when it was pointed out that acid hydrolysis of proteins in the presence of carbohydrates, which are inevitably found in all foodstuffs, results in the loss of a considerable portion of the protein nitrogen in the form of a black insoluble product known as "humin". The origin of humin nitrogen has been the subject of much study. Thus, Gortner and Blish (*10*) demonstrated that all the tryptophane is lost in this form. Tristram (*20*) and others (*19*) have reported the destruction of arginine, histidine, and lysine proteins through acid hydrolysis in the presence of carbohydrates. The loss of tyrosine, cystine, and methionine through the same mechanism has been demonstrated by Lugg (*14*). Kuiken and co-workers (*12*) have recently reported significant losses of valine, leucine, and isoleucine in casein when hydrolyzed in the presence of carbohydrates. In view of this evidence, it would appear impossible to secure accurate information on the amino acid composition of the vegetable by the direct hydrolysis technique.

The alternative solution of the problem lies in the isolation and purification of the protein moiety of the vegetable. Previous attempts at this approach have been notably unsuccessful.

The neutral saline extraction technique of Osborne (*17*), which proved so useful in his study of the seed proteins, was found ineffective when applied to fresh vegetables. The success of the maceration-extraction technique, also tried by Osborne and Wakeman (*18*), was hampered by filtration difficulties. In 1923, Chibnall (*7*) achieved the extraction of 40% of the total nitrogen of some leaf proteins by the aqueous ether treatment. However, he later found these preparations contaminated with pentosans and withdrew his analyses (*8*). Methods using combinations of various solvents (*11*) and enzymic removal of the carbohydrate (*9*) have been applied with some success to seed meals but are not readily adaptable to the study of fresh products. Mazur and Clarke (*15*) have shown in their study of the marine algae that a carbohydrate-contaminated preparation containing 60 to 90% of the total nitrogen could be conveniently obtained by extraction with 90% formic acid.

After numerous experiments with modifications of the various schemes suggested by these earlier attempts, the authors found that the carbohydrate contaminants of the formic acid extracts of the vegetables prepared as described by Mazur and Clarke could be quantitatively removed by the addition of ethanol without loss of nitrogen. The final product resulting from the isolation procedure evolved on the basis of this finding was found suitable for rat-feeding experiments or on acid hydrolysis for amino acid analysis and contained 90 to 95% of the total nitrogen of the fresh vegetable.

EXPERIMENTAL

SOLUBILITY OF PROTEINS AND CARBOHYDRATES IN FORMIC ACID AND FORMIC ACID–ETHANOL MIXTURE. Preliminary ex-

Table I. Solubility of Biological Products in 90 Per Cent Formic Acid

Class	Substance	Type of Compound	Solubility at 20-25° C. 90% formic acid	90% formic acid, 1 volume + 95% ethanol, 2 volumes
			Grams per 100 Cc.	
Carbohydrates	Cellulose	β-Glucosan	0.00	0.00
	Cornstarch	α-Glucosan	0.00	0.00
	Lintner starch	α-Glucosan (modified)	0.00	0.00
	Dextrins	Derived α-glucosan	0.13	0.00
	Sucrose	Disaccharide	5.60	4.50
	Dextrose	Monosaccharide	6.65	5.10
	Gum arabic	Pentosans + hexosans	0.00	0.00
	Gum tragacanth	Pentosans + hexosans	0.00	0.00
	Agar-agar	Galactans + mannans + fucosans	1.98	0.00
Proteins	Zein	Prolamin	7.25	7.25
	Gelatin	Albuminoid	5.90	5.90
	Hemoglobin	Chromo-histone	3.64	3.64
	Lactalbumin	Albumin	0.51	0.51
	Casein	Phosphoprotein	3.07	3.07

periments demonstrated that the formic acid extraction of the fresh vegetables after acetone fixation yielded high nitrogen recovery with an unavoidable impurity of a polysaccharide nature. The observation that the addition of 2 volumes of ethanol to 1 volume of formic acid extract of spinach leaves resulted in the quantitative precipitation of these carbohydrates with only a negligible loss of nitrogen prompted investigation of the solubility of some carbohydrates and proteins in formic acid and formic acid–ethanol mixture.

To 0.2- to 1.0-gram samples of various carbohydrates dried to constant weight in 15-cc. centrifuge tubes are added 10 cc. of 90% formic acid. After thorough mixing the tubes are stored in a 60° oven for 24 hours, being removed and shaken mechanically for 20 minutes at 10 intermittent intervals during the period. At the end of this time, the volume is adjusted to the original level and the tubes are centrifuged. The supernatant solution is decanted and saved and the residues are dried to constant weight in the vacuum desiccator over calcium chloride. The solubility of the carbohydrates per 100 cc. of formic acid is estimated by multiplying by 10 the weight loss incurred by the original sample (Table I). These values were checked by the weight of residues obtained by desiccation of the respective supernatant solutions. Of the carbohydrates tested only dextrose, sucrose, dextrins, and agar-agar are soluble in formic acid. The addition of 2 volumes of 95% ethanol to 1 volume of formic acid solutions of these four carbohydrates results in the quantitative precipitation of dextrins as agar-agar, but not of dextrose or sucrose.

The solubility of the proteins in formic acid is more conveniently estimated from the nitrogen content (N × 6.25) of the supernatant solutions obtained by application of the previously described procedure to protein samples. It is clear from the data so obtained (Table I) that these proteins are equally soluble in formic acid and the formic acid–ethanol mixture. Since the polysaccharides appear to be the principal carbohydrate contaminants of the fractions resulting from the formic acid extraction of the vegetables, the solubility of the proteins and insolubility of the polysaccharides in the formic acid–ethanol mixture affords a surprisingly simple method for the purification of the protein moiety.

Procedure for Extraction of Proteins from Fresh Vegetables. From 0.5 to 1 kg. of the fresh vegetable is frozen by storage in the freezing compartment of an electric refrigerator set at 0° C. This serves to break down the cell walls, thereby increasing cell permeability to solvents. The vegetable is then sectioned in preparation for sampling and extraction. (Leafy vegetables are sliced in 1-cm. cross sections; cabbages and beans are diced; potatoes, carrots, and turnips are shredded.) Six 100- to 200-mg. aliquots of this product are weighed out immediately on the torsion balance and total nitrogen is determined by micro-Kjeldahl method (*16*). Another 100-gram aliquot is set aside for the preparation of the alkaline hydrolyzate needed for estimation of tyrosine and tryptophane as described by Lugg (*13*).

The remainder of the prepared vegetable is placed in a 2-liter (0.5-gallon) food jar and covered with acetone, U.S.P., and after 2 days the acetone is removed by decantation and saved. The vegetable is then transferred to a fine-mesh cloth sack and submitted to a continuous acetone extraction for 24 hours in the modified Soxhlet apparatus shown in Figure 1, using the 600-watt hot plate, *A*. At the end of this time the acetone is removed from the extractor and combined with the first acetone extract. The acetone of the combined fractions is recovered by distillation, the insoluble coloring matter and lipids are filtered out, and nitrogen content of the aqueous residue is determined by micro-Kjeldahl method. This nitrogen which is extracted by the acetone treatment results from the mobilization of plant juices incident to dehydration and for want of a better term is called the acetone-soluble fraction. The low-protein nitrogen content of these fractions is indicated by the nitrogen content of precipitates obtained on the addition of 25 cc. of 10% trichloroacetic acid to 15 cc. of sample (Table II). Moreover, since 75 to 85% of the total nitrogen of these fractions occurs as α-amino nitrogen (*4*), the amino acids of these fractions are determined directly without hydrolysis and by special techniques to circumvent the interference of the carbohydrates present (to be published).

Since excessive decomposition of formic acid at its boiling point prevented the use of a continuous extraction technique, the acetone-extracted vegetable is treated as follows: The sack and contents are removed from the extraction chamber, *C*, and exposed to an air current for several hours to drive off the acetone. The incased vegetable is returned to the chamber, which is now filled with 90% formic acid to just below the discharging level. The temperature of the chamber is maintained at 75° to 80° C. by means of a 100-watt lamp, *B*, and the extraction is continued for 24 hours. This first portion of formic acid is discharged into the boiling flask by the addition of formic acid to a level above the siphon tube and the extraction continued for another 24 hours with a new portion of formic acid. This second formic acid extract is also discharged into the boiling flask. Two such operations suffice to extract almost all the available nitrogen.

The fibrous residue from both acetone and formic acid extractions is transferred to a 2-gallon enameled pail and mixed mechanically for 30 minutes with 2 liters of 95% ethanol to facilitate handling and subsequent drying of the product. The solid matter is removed by gravity and suction filtration, air-dried, and analyzed for nitrogen. The alcohol filtrate is made to 2 liters and added to the combined formic acid fractions which have been previously concentrated in vacuo to 1 liter. The mixture is then allowed to stand for 2 hours at room temperature and the precipitated carbohydrates are filtered out first by gravity through fluted paper and finally by suction in a Büchner funnel. The alcohol and formic acid of these filtrates are removed by distillation in vacuo. These residues were found to be uniformly carbohydrate-free by the Benedict, Molisch, and iodine tests and constitute the formic acid-soluble fraction of the vegetable nitrogen. They are suitable at this point for rat-feeding experiments. Or, they are made to 125-cc. volume with water and, after the addition of 75 cc. of concentrated hydrochloric acid, hydrolyzed by boiling under reflux in an all-glass apparatus for 24 hours. The excess acid is removed by concentrating the hydrolyzate in vacuo to a thick sirup three times successively after the addition of water. The final product is made to 250 to 300 cc. (pH 1 to 2), total nitrogen is determined, and then the humin is filtered out. The humin is extracted with 150 cc. of boiling water and the extract combined with the original filtrate. This final solution is concentrated in vacuo to 200 cc. and then submitted to nitrogen and amino acid analysis. The humin is air-dried and analyzed for nitrogen. A flow sheet of the procedure is given in Chart I.

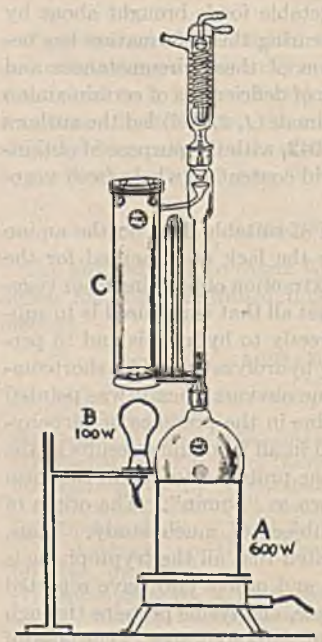


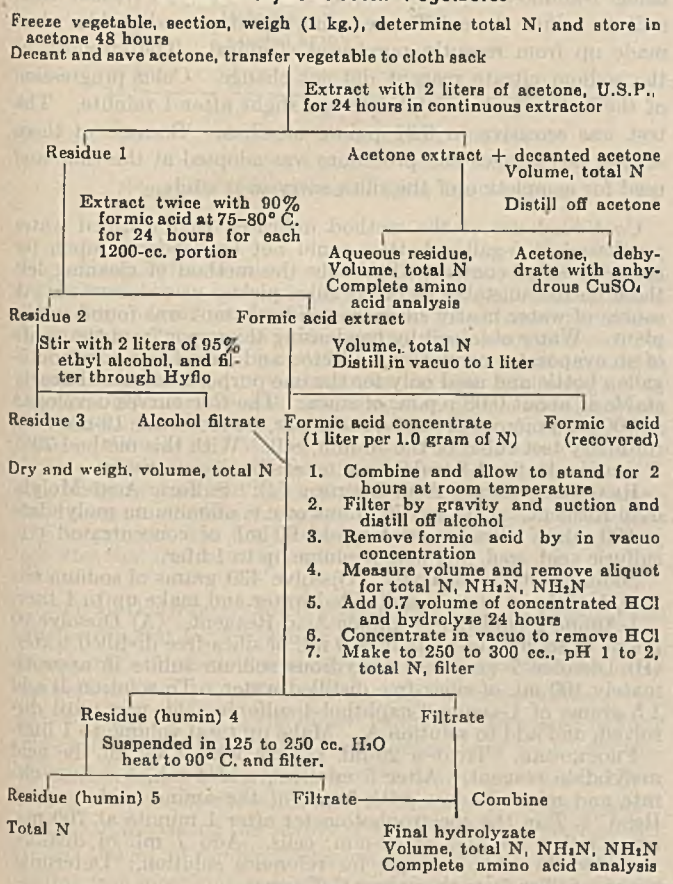
Figure 1. Extraction Apparatus

A, 600-watt hot plate; *B*, 100-watt electric lamp; *C*, extraction chamber

Table II. Distribution of Nitrogen in Fresh Vegetables

Vegetable	String Beans	Carrot	White Potato	White Turnip	White Cabbage	Chinese Cabbage	Kale	Spinach	Celery (Pascal)
Origin	Md.	Calif.	Idaho	N. J.	Md.	Ill.	Md.	N. Y.	Calif.
Sample analyzed, grams	828	1050	1044	890	791	888	590	1290	1278
Total N, grams	2.35	1.33	3.72	1.55	1.36	1.75	3.12	5.43	1.78
Protein (N × 6.25), % Found	1.78	0.79	2.24	1.08	1.07	1.23	3.29	2.63	0.87
U. S. Dept. Agr. (6)	2.4	1.1	2.0	1.1	1.4	1.4	3.2	2.3	1.0
Nitrogen extracted Grams	2.1	1.24	3.54	1.52	1.34	1.68	2.86	5.34	1.63
%	90.7	92.5	95.1	97.9	98.6	96.2	91.4	98.2	92.0
Acetone-soluble N, grams	0.57	0.24	1.32	0.33	0.37	0.63	0.48	1.10	1.09
Acetone-soluble N, % of total N	24.2	18.0	35.5	21.2	27.1	36.2	15.4	20.2	61.4
Protein-N in acetone-soluble fraction, mg.	13.2	14.7	22.0	13.6	31.0	19.3	5.3	24.1	8.4
Formic acid–soluble N, grams	1.54	0.99	2.22	1.19	0.97	1.05	2.36	4.24	0.54
Formic acid–soluble N, % of total N	66.5	74.5	59.6	76.7	71.5	60.0	76.0	78.0	30.6
Residue N, gram	0.249	0.095	0.183	0.025	0.018	0.063	0.261	0.093	0.154

Chart I. Flow Sheet of Nitrogen Extraction Procedure for Amino Acid Analysis of Fresh Vegetables



Application of the Method to Some Vegetables. The vegetables needed for this study were obtained from retail stores and only the edible portions used. The data on nitrogen distribution are recorded in Table II. Inasmuch as the protein content of the vegetables is a function of numerous variables, the protein content (calculated as N × 6.25) of the specimens is tabulated for comparison with that reported for the vegetable by the U. S. Department of Agriculture (*6*).

COMMENTS

The authors' data on the solubility of various carbohydrates in formic acid point to the dextrins and mucilages as constituting the principal impurities in the formic acid extracts of the vegetables. In 1939 circumstantial evidence led Lugg (*14*) to point out these substances as the possible contaminants in his leaf protein preparations. He was unable to remove them by isoelectric precipitation. It is clear from the present study that the solubility differences in the formic acid–ethanol mixture afford an effective means of separating these carbohydrates from the proteins. However, it is wholly probable that in the study of other vegetable foodstuffs the carbohydrate-protein relationships may be such as not to be separable by this technique.

The authors' experiences with the application of the formic acid–ethanol method to dried seed meals or pulverized dehydrated vegetables were unsatisfactory owing to the formation of gels and loss of dispersion of protein achieved in these preparations. Attempts to overcome these difficulties by the use of fillers such as Celite or filter paper were not completely successful. In the best experiments of this series only 60 to 70% of the total nitrogen could be removed after 5 extractions with formic acid. It appears, therefore, that the adaptability of the method to other foodstuffs is limited by the particle size and physical characteristics of the product.

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Determination of Soluble Silica in Very Low Concentrations

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A method for the determination of soluble silica in very pure central station steam or condensate is discussed applicable to the determination of silica in low concentrations (or in small samples) in any water. Its sensitivity allows Nessler tube comparison for values of silica as low as 0.02 p.p.m. A procedure for developing temporary molybdenum blue color standards of long stability is presented.

MODERN high-pressure central stations are equipped to supply steam of very high quality. Loss of turbine efficiency and capacity because of turbine blade deposits is therefore not expected in such installations. Nevertheless, many high-pressure plants have experienced blading deposits, which have often contained a high percentage of silica. The appearance of such deposits on the blades of a 1250-pound superposed turbine, as well as on the blades of the associated 300-pound units, at one station initiated tests to relate boiler water constituents to steam silica content. Obviously such a study necessitated very reliable determinations of soluble silica in dilute concentrations. The development of an acceptable silica determination procedure by some modification of published methods is shown in this paper.

SILICA BY SULFITE REDUCTION OF SILICOMOLYBDATE

The plant laboratory had for some time been using rapid colorimetric methods whenever possible for feedwater and boiler water control testing, a Coleman Universal spectrophotometer being utilized for the purpose. Silica in the boiler water was determined by the Kahler (*2*) method, measuring concentration of silica by means of the blue reduction color of silicomolybdate. With this procedure sulfite is used for reduction, and pH control is used to prevent phosphate interference. Concentration-transmittance curves for the method were developed at a wave length of 700 millimicrons with both 18-mm. and 40-mm. cell optical depths. The curves are linear, 70% transmittance being equivalent to 7.5 and 2.4 p.p.m., respectively.

To adapt the test method at hand to steam analysis, it was only necessary to broaden out the p.p.m. scale of the 40-mm. cell depth curve, making it readable to 0.1 p.p.m. of silica. This sensitivity appeared to be ample for the expected silica values in the steam. Distilled water for reagents was purchased outside the plant. It was assumed that very low concentrations of orthophosphate, with low concentrations of silica, would cause no interference. Hard-rubber bottles were used for all reagents. Pyrex 250-ml. glass-stoppered bottles were used for sample collection.

Subsequent use of the method brought out some practical difficulties. At this very low range of silica concentration the effect of color progression was found to be critical. The rapid color development required measuring the 1-minute sulfite reduction time with stop-watch accuracy. It became evident that the stable life of the reagents was short, the molybdate reagent being dependable for only 8 hours when used for determining small silica values. New bottles, or bottles freshly cleaned with dichromate cleaning solution, were found to cause erroneous silica determinations. Accordingly, separate bottles were provided to sample each point being tested and were used only for that purpose.

Reagents, Sulfite Method (*2*). Hydrochloric acid, 0.248 *N*; ammonium molybdate solution, 102 grams per liter; and sodium sulfite, 170 grams per liter.

Procedure. Treat a 10-ml. sample with 5 ml. of acid and 5 ml. of ammonium molybdate solution. Reduce within 1 to 5 minutes with 10 ml. of sodium sulfite solution. Read % *T* in spectrophotometer 1 minute after reduction at 700 mμ with 18-mm. or 40-mm. cell. Use sample reference solution containing the acid and sulfite plus 5 ml. of distilled water. Determine p.p.m. of silica from the proper *C-T* curve.

SILICA BY AMINO ACID REDUCTION OF SILICOMOLYBDATE

As the carry-over study progressed, values of less than 0.1 p.p.m. of silica became important. It was learned that an increase of the sample volume to 50 ml. made the determination more sensitive, and that the method used by Lindsay and Bielenberg (*4*) resulted in still greater sensitivity. This latter method prevents phosphate interference by destroying the phosphomolybdate complex with sodium citrate. A mixed sulfuric acid and ammonium molybdate reagent proved to be stable. The blue reduction color was obtained by using 1-amino-2-naphthol-4-sulfonic acid reagent rather than sodium sulfite alone. The reducing agent proved stable when made up from recently purchased chemical. The strength of the sodium citrate reagent did not change. Color progression of the reduced silicomolybdate was slight after 1 minute. The test was sensitive to 0.01 p.p.m. of silica. Because of these several advantages the procedure was adopted at this time and used for completion of the silica carry-over study.

Continued use of the method indicated that distilled water purchased in 5-gallon bottles could not be depended upon for constant silica content. Probably the method of cleaning left these bottles unstable as far as silica pickup was concerned. A source of water nearly silica-free and constant was found in the plant. Water obtained by condensing the vapor from the vents of an evaporator condenser, collected and stored in a common 5-gallon bottle and used only for the one purpose, remained nearly stable at about 0.03 p.p.m. of silica. The *C-T* curves developed at 700 millimicrons are not quite linear, for either the 19-mm. cell (ordinary test tube) or the 40-mm. cell. With this method 70% *T* is equivalent to 1.8 and 0.7 p.p.m. of silica, respectively.

Reagents, Amino Acid Method (*4*). Sulfuric Acid–Molybdate Reagent. Dissolve 75 grams of C.P. ammonium molybdate in 800 ml. of silica-free water, add 60 ml. of concentrated C.P. sulfuric acid, cool, and make volume up to 1 liter.

Sodium Citrate Reagent. Dissolve 430 grams of sodium citrate, U.S.P., in silica-free distilled water and make up to 1 liter.

1-Amino-2-naphtho-4-sulfonic Acid Reagent. (A) Dissolve 90 grams of sodium bisulfite in 800 ml. of silica-free distilled water. (B) Dissolve 7 grams of anhydrous sodium sulfite in approximately 100 ml. of silica-free distilled water. To solution B add 1.5 grams of 1-amino-2-naphthol-4-sulfonic acid, mix until dissolved, and add to solution A. Make up total volume to 1 liter.

Procedure. Treat a 20-ml. sample with 2 ml. of the acid molybdate reagent. After 5 minutes, add 4 ml. of sodium citrate and mix. Reduce with 1 ml. of the amino acid reagent. Read % *T* in the spectrophotometer after 1 minute at 700 mμ, using either 19-mm. or 40-mm. cells. Add 7 ml. of distilled water to 20 ml. of sample for reference solution. Determine p.p.m. of silica from the proper *C-T* curve.

RECENT MODIFICATIONS

During the carry-over study it became evident that the sensitivity of the test could be increased. Straub (*9*) had obtained better sensitivity by maintaining a low pH. The sensitivity of the authors' test was increased by omitting the sodium citrate in phosphate-free samples. A check on the method showed a sample pH of 1.96 after the addition of sulfuric acid–molybdate reagent, and 5.0 after the addition of sodium citrate. Since the test appeared so desirable in many respects, an attempt to improve the sensitivity seemed in order.

The literature revealed several pertinent facts. Knudson, Juday, and Meloche (*3*) show maximum development of the

yellow silicomolybdate at a pH between 1.6 and 2.0 using sulfuric acid. They verify the mole ratio as being 1 SiO_2 to 12 MoO_3, and establish that a relatively small excess of molybdate is necessary to ensure completion of the silicomolybdate reaction. These authors use a 100-ml. sample and measure values as low as 0.1 p.p.m of silica by comparing the yellow color developed.

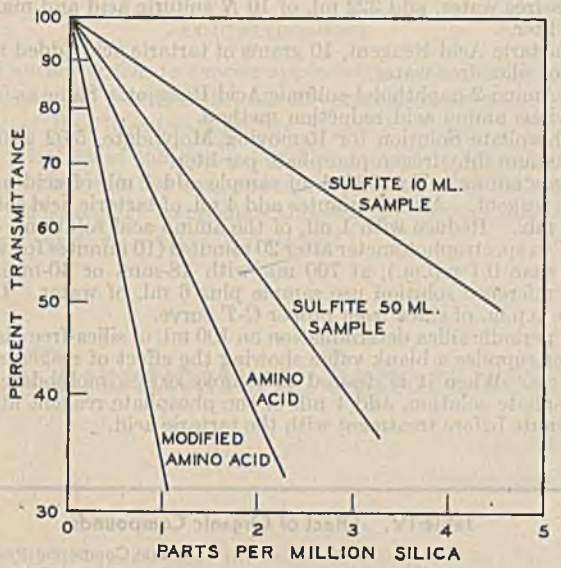

Figure 1. Comparison of Silica Test Methods

The silicomolybdate reaction is indicated as being complete within 5 minutes. Schwartz (*6*) measures the yellow silicomolybdate color and uses oxalic acid to prevent phosphate interference. He indicates the reaction in destroying phosphomolybdate to be 1 MoO_3 to 1 $(COOH)_2$. Straub (*9*) also has used oxalic acid in a reduction method. A statement in the bibliography by Schwartz (*7*) indicates that tartaric acid or citric acid will prevent phosphate interference. In their work on the molybdenum blue reaction, Woods and Mellon (*11*) show that in some cases the sulfuric acid–molybdate reagent is superior to hydrochloric acid–molybdate reagent and that chlorostannous acid is a superior reducing agent. Snell (*8*) suggests the use of excess phosphoric acid to prevent iron and phosphate interference. Thayer (*10*) indicates that iron must be removed.

From all this information it appeared that a very practical and satisfactory test for low values of silica might be evolved. The phosphoric acid method was eliminated as impractical for rapid routine work. A 100-ml. sample was chosen since the effect of reagents would be less in the larger volume. The mixed sulfuric acid–molybdate reagent was retained because of its known stability and its acceptance by some investigators. A pH check and calculations of the silica-molybdate ratio indicated the advantage of increasing the acidity of this reagent to allow use of 1 ml. per 100 ml. of sample. Because of its expected value in preventing iron interference, tartaric acid was chosen as the reagent to destroy phosphomolybdate, the strength and amount of this reagent to supply an excess being calculated from the relationship 1 MoO_3 to 1 $(COOH)_2$. Four milliliters of a 10% solution were found to supply sufficient excess and also to maintain proper pH. One milliliter of the amino acid reagent supplied ample reductant.

Concentration-transmittance curves were developed for this method. For both the 18-mm. and 40-mm. optical depths they are linear for the low values being investigated. With this method, 70% *T* is equivalent to 0.85 and 0.33 p.p.m. of silica, respectively.

The sensitivity of the chlorostannous acid reductant was checked upon by developing a second 40-mm. optical depth *C-T* curve, the amino acid reductant being replaced by the chlorostannous acid as used by Woods and Mellon (*11*), which was found to be slightly more sensitive. However, the amino acid was chosen over the chlorostannous acid because of its greater stability.

A silica-free water is required for accurate silica *C-T* standards and for making up reagents. Distillation of turbine condensate in a small laboratory still with subsequent storage in a tin-lined tank now supplies a high-grade product. A blank reading for reagents, kept in Pyrex bottles, has not exceeded 0.025 p.p.m. of silica for this modified method. As yet no limit has been found for length of reagent life, reagents as old as 4 months have not exceeded the above blank value. The marked increase in sensitivity of the modified method over previous methods is shown in Figure 1.

Table I. Standard Solution Comparison

Origin of Standard	Comparison	P.p.m. Theoretical	% *T*	P.p.m. from Gravimetric Curve	% Deviation from Gravimetric Curve
A.P.H.A. colorimetric, 9-23-41	10 ml. of sulfite	1.0	87.0	0.9	−10
Same, 9-30-42	10 ml. of sulfite	1.0	87.0	0.9	−10
Gravimetric[a]	10 ml. of sulfite	1.0	86.0	1.0	...
Gravimetric	Amino acid	1.0	61.0	1.0	...
Purchased[b] 6-8-43	Amino acid	1.0	61.0	1.0	0
Same, 1-2-44	Amino acid	1.0	61.0	1.0	0
Same, 1-2-44	Modified	0.40	65.2	0.40	...
$Na_2SiO_3.9H_2O$ C.P., fresh, 1.255 grams + 1 gram of NaOH/250 ml.	Modified	0.40	63.2	0.43	+ 7.50
Same, old	Modified	0.40	63.5	0.425	+ 6.25
Na_2SiO_3, commercial[c], 0.5083 gram + 1 gram of NaOH/250 ml.	Modified	0.40	64.5	0.41	+ 2.50

[a] In hard-rubber container.
[b] W. H. & L. D. Betz, rosin-lined container.
[c] Cowles Detergent Co. (Drymet), barrel previously opened.

EFFECTS OF IONS AND CONDITIONS ON THE MODIFIED METHOD

Orthophosphate may be present in steam as a result of some boiler operating disturbance. It was found that tartaric acid entirely prevents interference of this ion in any concentration up to the theoretical limits of the reagents. Other organic acids, such as citric or oxalic, are known to be equally effective.

The rate of color development after reduction is shown in Figure 2. It is apparent that the time required for complete color development increases with concentration. Twenty minutes is considered ample time within the proper temperature range. A *C-T* curve made up without regard to complete color development will tend to deviate from the linear.

Silica standard solutions were investigated to determine their accuracy and stability. Solutions used in developing the preceding curves, one made as described in the literature (*11*) and another made from a commercial anhydrous sodium metasilicate, are compared in Table I. The solution standardized by the A.P.H.A. method (*1*) using the chromate standards was found somewhat low. The nonhydrate ($Na_2SiO_3.9H_2O$), whether fresh or old solid, was found to deviate considerably from the theoretical value. The other solutions were acceptable as standards. Solutions have been found stable in a hard-rubber container for at least one year and in rosin-lined containers for at least 7 months. Investigators have shown (*5*) that silica solutions thus stored will not deteriorate.

Variations in sample temperature were found permissible in a range at least 10° plus or minus a normal 75° F. laboratory temperature. The temperature effect is shown in Table II.

Iron, present in high quality steam and condensate, is normally in concentrations less than 0.1 p.p.m. Neither ferrous nor ferric iron alone causes interference in this modified procedure. The effect of iron alone, iron with phosphate, iron with silica,

Table II. Effect of Sample Temperature

Sample Temperature °F.	Theoretical P.p.m.	Curve P.p.m.	Difference P.p.m.
62	0.200	0.192	−0.008
69	0.200	0.192	−0.008
84	0.200	0.204	+0.004
92	0.200	0.213	+0.013
67	0.100	0.084	−0.016
73	0.100	0.100	0
93	0.100	0.085	−0.015
93	0.050	0.048	−0.002
68	0.050	0.040	−0.010

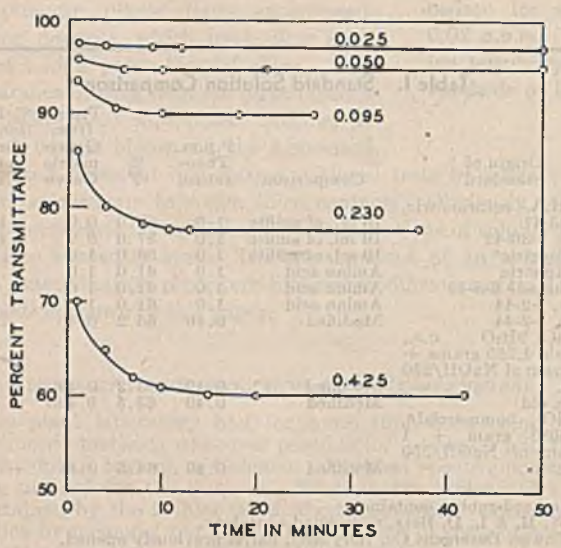

Figure 2. Color Development at Varying P.P.M. Concentration

and iron with both phosphate and silica is shown in Table III. The combination of ferrous iron and phosphate cannot be tolerated since the iron reduces any phosphomolybdate that is formed. The presence of this combination in steam or condensate is unlikely.

The effect of organic compounds was investigated because of academic interest. The highly colored nature of these compounds makes it important that the blank reference consisting of 100 ml. of sample and 6 ml. of distilled water be used. As may be seen in Table IV, no practical interference from the organic compounds investigated is then evident.

Table III. Effect of Iron and Orthophosphate

Silica as SiO_2			Combination Permissible	Ferric Iron	Ferrous Iron	Phosphate
Present P.p.m.	Found P.p.m.	Difference P.p.m.		Present P.p.m.	Present P.p.m.	Present P.p.m.
0	0	0	Yes	...	0.5	...
0	0	0	Yes	...	1.0	...
0	0	0	Yes	...	2.5	...
0	0	0	Yes	0.5	...	...
0	0	0	Yes	1.0	...	...
0	0	0	Yes	2.5	...	...
0.1	0.1	0	Yes	1.0	...	...
0	0	0	Yes	1.0	...	0.7
0.1	0.1	0	Yes	0.5	...	...
0.1	0.1	0	Yes	...	...	0.7
0.1	0.1	0	Yes	...	...	1.4
0.2	0.2	0	Yes	0.5	...	1.4
0	0	0	Yes	0.5	...	1.4
0	0.1	0.1	No	...	1.0	0.7
0.05	0.09	0.04	No	...	1.0	0.7
0.1	0.1	0	Yes	...	1.0	...
0.2	0.2	0	Yes	...	1.0	...

MODIFIED METHOD REAGENTS. Sulfuric Acid Molybdate Reagent. To 75 grams of ammonium molybdate dissolved in silica-free water, add 322 ml. of 10 *N* sulfuric acid and make up to 1 liter.

Tartaric Acid Reagent, 10 grams of tartaric acid added to 100 ml. of silica-free water.

1-Amino-2-naphthol-4-sulfonic Acid Reagent. Same as for the previous amino acid reduction method.

Phosphate Solution for Removing Molybdate, 5.02 grams of potassium dihydrogen phosphate per liter.

PROCEDURE. To 100 ml. of sample add 1 ml. of acid-molybdate reagent. After 5 minutes add 4 ml. of tartaric acid solution and mix. Reduce with 1 ml. of the amino acid solution. Read % *T* in spectrophotometer after 20 minutes (10 minutes for values less than 0.1 p.p.m.), at 700 mμ with 18-mm. or 40-mm. cell. For reference solution use sample plus 6 ml. of water. Determine p.p.m. of silica from proper *C-T* curve.

A periodic silica determination on 100 ml. of silica-free distilled water supplies a blank value showing the effect of reagents.

Note. When it is desired to remove excess molybdate with phosphate solution, add 1 ml. of the phosphate reagent at least 1 minute before treatment with the tartaric acid.

Table IV. Effect of Organic Compounds

Silica			Organic Compound Present	
			Lignin derivative	Chestnut tannin
Present P.p.m.	Found P.p.m.	Difference P.p.m.	P.p.m.	P.p.m.
0	0.01	0.01	10.0	..
0	0	0	..	5.0
0	0.01	0.01	..	10.0
0.05	0.05	0	0.1	..
0.05	0.055	0.005	..	0.1
0.20	0.20	0	0.1	..
0.20	0.20	0	5.0	..
0.20	0.205	0.005	10.0	..
0.20	0.20	0	..	0.1
0.20	0.20	0	..	5.0

NESSLER TUBE COMPARISON

The sensitivity of the modified test allows color matches as low as 0.02 p.p.m. in 50-ml. Nessler tubes. An attempt to prolong the life of temporary Nessler tube standards has proved effective. The method consists of removing, after the silica reaction is complete, all the active molybdate in the treated sample as phosphomolybdate and then destroying the phosphomolybdate with the organic acid being used for that purpose. The resulting molybdenum blue is identical with that obtained when excess molybdate is present. Standards in stoppered 50-ml. Nessler tubes remain stable for long periods. Tubes kept at room temperature, exposed to the sunlight part of the day, are stable at this writing, 18 days after treatment. The tubes are 18 mm. in diameter, permitting comparative readings directly in the spectrophotometer. Visual checks against fresh standards indicate stability to the eye as well as by instrument.

Note: Since the preparation of the original manuscript the molybdenum blue color standards thus developed have been found satisfactory for phosphate color standards as well as for silica standards when kept in sealed comparison bottles.

CONCLUSION

The modified amino acid reduction method for silica determination has proved very satisfactory and highly practical for routine power plant testing of high quality steam. The stability of the color allows samples to be treated in rapid succession and then all measured in a series later. There need be little regard for time after the required 20 minutes for maximum reduction.

The sensitivity of the method and long color stability after reduction allow Nessler tube comparison outside the laboratory. The proper preparation and storage of silica-free water is essential when testing for low silica concentration. Test conditions are not critical and no practical interference from ions other than silica has been observed.

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Quantitative Separations with an Exchange Adsorber

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A SIMPLE quantitative procedure has been devised to separate cations from anions in water solution with a solid ion-exchange adsorber. Separations that demonstrate the possibilities of the method have been made with a precision and accuracy as quantitative as the methods of determination.

The purpose of this work was an examination of the many adsorbers readily available for possible use in analytical chemistry, development of a method for the quantitative separation of inorganic cations from anions in water solution, application of this method to several kinds of cations and anions, and a brief examination of its limitations.

A typical application of the method would be the separation and subsequent determination of copper and sulfate in a solution of copper sulfate. An equation for such a separation would be:

$$2H^+A' + Cu^{++} + SO_4'' \rightleftarrows Cu^{++}A_2' + 2H^+ + SO_4''$$

where A′ is one equivalent of adsorber. The $Cu^{++}A_2'$ quantitatively separates the copper from the sulfate. The adsorber is washed with water and the sulfate determined in a solution containing only one cation, hydrogen ion. The copper is removed from the adsorber with hydrochloric acid and determined in the resulting solution.

EXPERIMENTAL

A suitable exchange adsorber for quantitative separations must be a reagent chemical. It should also have the following properties: a large adsorptive capacity per gram of adsorber, a rapid exchange of substances between adsorber and surrounding solution, stability, both chemical and mechanical, rapid and quantitative removal of unadsorbed substances with a small volume of wash solution, and rapid quantitative removal of adsorbed substances.

Analytical separations of sodium (*5*, *7*, *8*), copper (*8*), iron (*5–8*), calcium (*5–8*), chloride (*6*, *8*), sulfate (*4*, *5*, *7*, *8*), and phosphate (*6*, *8*), included in this work have been made by a similar method. The general properties and uses of many available adsorbers have been described (*2*, *9*).

Many experiments showed Zeo-Karb to be a satisfactory adsorber. The commercial adsorber obtained from the Permutit Company has many common cations on it, especially sodium ion, and it also contains sulfate ion. These must be removed and the adsorber prepared as a reagent chemical with hydrogen ion as the adsorbed cation. This is done by placing the adsorber in the apparatus described below and applying operation 4 for several hours.

SEPARATION APPARATUS. The apparatus shown in Figure 1 was designed and developed from work with a 0.1 *N* solution of ferric chloride and proved satisfactory for other separations. Twenty grams of dry adsorber were wet with water and placed on a mat of glass wool in the primary adsorber, *P*, with the tube, *E*, in the center of the adsorber. Five grams of adsorber were similarly placed in the secondary adsorber, *S*. The solutions were run through *P* and *S* with gravity flow and the apparatus is designed to adjust the rate of flow and time of contact to give the desired quantitative separations. Three apparatus were used.

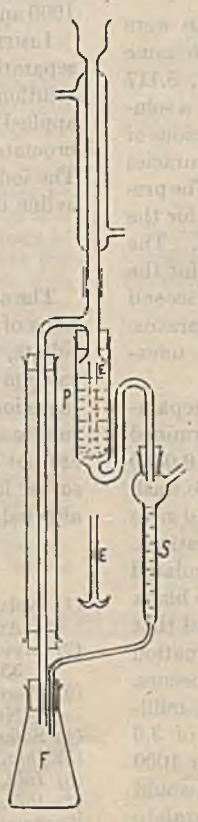

Figure 1

PROCEDURE FOR SEPARATIONS. Each separation began with reagent adsorber with only hydrogen ion adsorbed on it.

1. The apparatus was dismantled and washed with water to remove hydrochloric acid, then 100 ml. of water were allowed to run through *P* and 50 ml. of water through *S*. *P* and *S* were connected and 200 ml. of water were run through *P* and *S* as fast as it ran through *S*. Water was added to *P* just to cover the adsorber and then a little more was added to cause siphoning. A volume of 5 to 7 ml. was added each time.
2. A 25-ml. portion of solution was measured in a pipet and 50 ml. of water were added to it. The solution was added to *P* and allowed to run through *P* and *S* as in operation 1. The volume of the pipet used was 24.91 ml.
3. Adsorbers *P* and *S* were then washed with 200 ml. of water as in operation 1. The washings from 2 and 3 were collected together to determine the anion.
4. Twenty-five milliliters of 6 *N* hydrochloric acid were placed in the 250-ml. flask, *F*, and all parts of the apparatus were connected. The hydro-

chloric acid was distilled through *P* and *S* for 2 hours. The cation was determined in the solution in flask *F*. Three hours were required to make a separation; for 2 of the 3 hours no personal attention was required.

Reagents. Analytical reagent chemicals were used and potassium dichromate, sodium oxalate, ammonium dihydrogen phosphate, calcium carbonate, and boric acid were further purified by crystallization.

Separations. The procedures for the determinations were adapted from textbooks (*1*, *3*). Solutions of ferric chloride, copper sulfate, sodium oxalate, ammonium dihydrogen phosphate, and calcium borate were prepared of determinate or determined concentration. The ions of each solution were separated by the above procedure and the quantity of each ion was determined after the separation. Four or more determinations of each ion were made where necessary in each solution before the separations and the average values were considered the correct values. The precision of the results is the difference between the extreme values over the average value in parts per thousand and the accuracy is the difference between a determined value and the correct value over the correct value in parts per thousand. The results of the separations are given in Table I.

DISCUSSION OF RESULTS

The first three results of Table I were secured in an apparatus that had been used many times. The precision of these results for iron, 0.8 part per 1000, compares favorably with the precision of the determinations before the separations, 1.6 parts per 1000. The accuracy is +0.4 to +1.2 parts per 1000. The first three results for chloride have a precision of 0.5 part per 1000 compared with 1.4 parts per 1000 for the original solution. The accuracy of the chloride results is +5.0 parts per 1000. These high results appear to be a property of the apparatus and are not due to incomplete washing. The other results for iron and chloride from the same original solution were obtained from an apparatus that had been used very little. These and many similar results show that a seasoned apparatus is needed to give consistent results of sufficient precision and accuracy.

Two approximately 0.2 *N* solutions of copper sulfate were used. The amount of copper was determined and the same amount of sulfate was assumed present. One solution, 5.117 milliequivalents, gave the first four results in the table and a solution of 5.079 milliequivalents gave the other results. Precisions of the copper results are 1.8 and 1.2 parts per 1000 and the accuracies are −1.8 to 0 and 0 to +1.2 parts per 1000, respectively. The precisions of the results for sulfate are 4.3 parts per 1000 for the first solution and 7.8 parts per 1000 for the second solution. The accuracy of these values is +1.4 to −2.9 parts per 1000 for the first solution and +4.1 to −3.7 parts per 1000 for the second solution. This prevents the use of a constant for the apparatus. The adsorber contains sulfate, so such accuracy is not unexpected.

The determinations of sodium and oxalate after the separations required blanks. The blank for sodium was determined by following the entire procedure and gave an average of 0.0380 milliequivalent of sodium from eight determinations. The wash solution from operation 1 was used for the oxalate blank and gave an average of 0.0220 milliequivalent from nine determinations. The sodium oxalate solution was determinate and the calculated milliequivalent of sodium was 2.492. This shows that the blank for sodium is too small by about 0.014 milliequivalent and that the sodium removed from the adsorber by a blank determination is not the same as the sodium removed when an exchange occurs. The error for sodium is calculated by considering 2.492 milliequivalents the correct value for sodium. A precision of 3.6 parts per 1000 and an accuracy of +3.6 to +7.2 parts per 1000 are obtained. A blank of 0.052 milliequivalent of sodium would give equal precision and accuracy. The errors for the oxalate determinations are calculated from the average value, 2.492 milliequivalents, obtained by comparing the permanganate and oxalate solutions. The precision and accuracy for all the oxalate results are less than 2.0 parts per 1000.

The ammonium dihydrogen phosphate solution was determinate but determined values for ammonium and phosphate were obtained and used as the correct values. The calculated value for milliequivalents of ammonium ion is 2.491 and the determined value is 2.489. The values obtained after the separations show a precision of 1.2 parts per 1000 and an accuracy of +6.0 to +7.2 parts per 1000. This indicates that a constant of 0.016 milliequivalent should be applied and results would be equally precise and accurate. The calculated value for phosphate is 2.492 milliequivalents and the determined value is 2.497 milliequivalents. The precision is 3.6 parts per 1000 and the accuracy is +1.2 to −2.4 parts per 1000.

Table I. Milliequivalents of Ions Found after Separations

(Present in original solution: iron 2.549, chloride 2.018 milliequivalents)

Fe	Cl	Cu	SO_4	Na	C_2O_4	NH_4	PO_4	Ca	BO_3
2.552	2.027	5.108	5.124	2.506	2.488	2.505	2.500	2.486	2.491
2.550	2.028	5.108	5.107	2.509	2.491	2.504	2.494	2.486	2.489
2.551	2.027	5.117	5.102	2.506	2.490	2.504	2.495	2.494	2.491
2.562	2.018	5.117	5.106	2.501	2.490	2.507	2.496	2.495	2.489
2.558	2.037	5.079	5.097	2.510	2.490	2.504	2.492	2.486	2.491
...	2.032	5.085	5.100	...	...	2.505	2.491	2.494	2.490
...	...	5.079	5.060	...	...	...	...	2.494	2.491
...	...	5.085	5.064	...	...	...	...	...	...

Determinate 0.1 *N* calcium borate, 0.1 *N* calcium chloride, and 0.1 *N* boric acid solutions were prepared and the comparison of the two latter solutions with solutions of potassium permanganate and carbon dioxide–free sodium hydroxide, respectively, gave determined values for the original solutions to compare with values after the separations: 2.492 milliequivalents of calcium and 2.492 milliequivalents of borate. Precision of the calcium results is 3.6 parts per 1000 and the accuracy is +1.2 to −2.4 parts per 1000. Precision of the borate results is 0.8 part per 1000 and the accuracy is −0.4 to −1.2 parts per 1000.

Limitations of the Method. Conditions for a successful separation are a small total ion concentration and a pH of the solution not less than 2 and preferably about 4. This procedure applied to 0.1 *N* solutions of potassium dichromate, potassium bromate, and potassium iodate gave reduction of the anions. The iodide in a 0.1 *N* potassium iodide solution was oxidized to iodine on the adsorber.

SUMMARY

The adsorber Zeo-Karb used in a simple apparatus gave separations of cations and anions in solutions of ferric chloride, copper sulfate, sodium oxalate, ammonium dihydrogen phosphate, and calcium borate as quantitative as the methods used to determine these ions with the exception of sulfate ion. The separations of sulfate are quantitative enough to be useful when other methods are not available. The acid and reducing properties of the adsorber limit the usefulness of this method to ions not adversely affected by these properties.

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Substitution of Bromine

When Determining Unsaturation of Straight and Branched-Chain Olefins

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Substitution (evidenced by formation of hydrobromic acid) has been found to occur for a variety of olefins including isobutylene polymers, pentene-2, hexenes, and octene-1, when bromate-bromide reagents are employed for determining unsaturation. Halohydrin formation with generation of an equivalent amount of hydrobromic acid is negligible if the reagents contain a high concentration of bromide ion. Bromine numbers approaching theory have been obtained on some of the compounds studied, such as diisobutylene and trimethylethylene, even though substitution occurs. This is possible only if the departure from the expected reaction is automatically compensated. The bromine substitution product seems to be relatively indifferent to further bromination either by substitution or by addition. While bromine numbers obtained on certain petroleum fractions may be somewhat misleading, the method is still recommended for general analysis until a more direct method is found.

IN A previous article (*9*) the authors presented data showing that the theoretical bromine number of diisobutylene and trimethylethylene could be obtained by employing a modification of the Francis method (*2*). The modification failed, however, to give theoretical values for the higher polymers, tri- and tetraisobutylene. Since these data were published, a study has been made to determine whether other procedures would give reliable unsaturation values for all three isobutylene polymers.

The procedures investigated were based on addition of bromine and thiocyanogen iodide to the double bond, and included those of McIlhiney (*10, 11*), Kaufmann and Grosse-Oetringhaus (*7*), Uhrig and Levin (*13*), and certain unpublished modifications of the Lewis and Bradstreet method. All these methods gave inconsistent results, and in all cases except the thiocyanogen iodide procedure extensive formation of hydrobromic acid occurred. The formation of halogen acid is in line with the observations of previous investigators.

Hal'pern (*4*) states that bromometric analysis by the methods of McIlhincy and of Kaufmann (*6, 8*) is usually accompanied by evolution of hydrogen bromide, although the main mass of this acid is not necessarily the result of substitution. In a study of pinene with Kaufmann's reagent, Hal'pern concluded that the primary products of interaction of unsaturated compounds with bromine are not 1,2-dibromides. He considers them to be unstable bromides which are rearranged immediately to form the stable dibromides or are decomposed into hydrogen bromide and unsaturated monobromides, with the possibility of the formation of a new compound that would be incapable of adding bromine.

Kaufmann (*6, 8*) utilized a solution of bromine in methanol saturated with sodium bromide for determining bromine numbers of various organic compounds. In verifying the method by determining the total amount of bromine consumed he found the values obtained were always the same as those calculated and he, therefore, assumed that the double bond was saturated without the formation of substitution products. Contrary to such a supposition, Jordan (*5*) found when titrating styrene and indene by the same method that one half of the bromine consumed was present in the bromination flask as hydrobromic acid.

Francis (*3*) reported that saturation of double bonds with bromine water gives principally the bromohydrin derivatives and that the relative amount of dibromide formation is just as small with bromine water in 4 *N* sulfuric acid, in which the concentration of hypobromous acid is negligible, though the rate of saturation is less than one thousandth as great.

Terry and Eichelberger (*12*) have shown that bromohydrin formation can be prevented in the case of sodium maleate and sodium fumarate by a high concentration of sodium bromide.

Buc (*1*) has reported that in the case of highly branched olefins in the higher molecular weight ranges, the major reaction with halogens in general consists of substitution. He employed reagents saturated with potassium bromide.

Recent data obtained on a number of straight- and branched-chain olefins (including diisobutylene and pentene-2) show that halohydrin formation with generation of an equivalent amount of hydrobromic acid does not occur to any appreciable extent when reagents saturated with potassium bromide are used for measuring unsaturation. It is believed that the major amount of hydrobromic acid found in the following experimental work is due to substituted halogen in the molecule. For the sake of consistency it is thus preferred to use the term "substitution" to account for the total amount of halogen acid determined in each experiment.

It is obvious that in cases where substitution occurs to any appreciable extent, a measure of unsaturation based on bromine addition will not be reliable. In view of the apparent occurrence of both addition and substitution, a quantitative study has been made of the extent to which each takes place to determine whether conditions exist which will permit substantially complete addition before appreciable substitution occurs.

DEFINITIONS

In treating the experimental data, it is convenient to consider three bromine numbers (apparent, substitution, and addition) having the following definitions:

1. Apparent bromine number is defined as the total weight of bromine consumed when employing a bromine addition method. The apparent bromine number is equivalent to:

$$\frac{(\text{Total weight of bromine added} - \text{unreacted bromine})}{\text{weight of sample}} \times 100$$

$$RCH{=}CHR' + Br_2 \rightarrow RCHBr{-}CHR'Br$$

2. Substitution number is defined as the weight of bromine contained in the hydrobromic acid formed during the addition reaction. The substitution number may be expressed as:

$$\frac{\left(\text{Weight of hydrobromic acid} \times \frac{Br}{HBr}\right)}{\text{weight of sample}} \times 100$$

3. Addition number is defined as the weight of bromine that is added to a double bond when determining unsaturation by a bromate-bromide method. The addition number is equivalent to:

Apparent bromine number $-$ $2x$ substitution number

or

$$\frac{[\text{Total weight of bromine consumed} - 2x\ (\text{bromine equivalent to hydrobromic acid})]}{\text{weight of sample}} \times 100$$

All three numbers have the dimensions $\frac{\text{Cg. of bromine}}{\text{Grams of sample}}$

The experimental work consists of a study of addition and substitution of bromine occurring when nascent bromine generated from a bromate-bromide solution is employed. The reactions were carried out under varying conditions described in this paper.

Table I. Addition and Substitution by Bromate-Bromide Method without Excess Acid

Compound	Theoretical Bromine No.	Apparent Bromine No.	Substitution No.	Bromine Consumed during Substitution Reaction	Addition No.	Temperature
	Cg./g.	*Cg./g.*	*Cg./g.*	*Cg./g.*	*Cg./g.*	*° C.*
Diisobutylene	142.4	123.1	57.7	115.4	7.70	25
		127.4	60.1	120.2	7.14	25
Triisobutylene	94.9	49.5	19.3	38.6	10.97	25
		43.3	18.9	37.8	5.62	25
Tetraisobutylene	71.2	45.8	19.2	38.4	7.40	25
		56.0	23.6	47.2	8.80	27

INVESTIGATION OF SUBSTITUTION WITH BROMATE-BROMIDE REAGENT

It had been suspected for some time that considerable substitution occurred when the unsaturation of highly branched olefins was measured by the authors' method previously published (*9*). The extent of substitution and addition of bromine has been examined when various modifications of the method have been applied to measurements of unsaturation in isobutylene polymers.

Use of Equivalent Amount of Sulfuric Acid. The following experiment was conducted to find out if the apparent bromine number could be obtained without the formation of hydrobromic acid by using an exactly equivalent amount of sulfuric acid necessary for completion of the reaction instead of an excess as directed in the original procedure (*9*). The compounds used were di-, tri-, and tetraisobutylene.

Procedure. Twenty milliliters of saturated potassium bromide solution and 15 ml. of *n*-heptane are placed in a 250-ml. glass-stoppered flask, and 1 ml. of sample is added. Sufficient 0.5 *N* potassium bromate for the sample plus 1.2-ml. excess is next added, followed by an exactly equivalent amount of 0.5 *N* acid. The flask is shaken vigorously for 2 minutes, saturated potassium iodide solution added, and the liberated iodine titrated with 0.1 *N* thiosulfate, using the disappearance of the iodine color as an indicator. This titration represents the free bromine. Saturated potassium iodate solution is now added, and any iodine present titrated with thiosulfate. The amount of iodine liberated is equivalent to the hydrobromic acid formed. Apparent addition and substitution bromine numbers are calculated as shown above.

The data given in Table I indicate not only that the apparent bromine number is too low, but also that substitution occurs when excess acid is not present.

Effect of Amount of Bromate Solution on Apparent Bromine Number. It had been noted in the original method (*9*) that after addition of a few milliliters of bromate (2 to 4 ml., or a fraction of the volume necessary for theoretical requirements) to samples of isobutylene polymers and subsequent shaking, a faint yellow color was evident. If addition of the reagent was stopped at this point and the sample was shaken for 2 minutes, the yellow color often persisted, indicating an apparent excess. Heretofore the procedure has been to ignore this slight color and continue to add bromate until a strong brownish yellow color is obtained before shaking for the specified 2 minutes. However, in the actual determination of unsaturation in the compounds, for this study, addition of bromate was purposely stopped at the first sign of the yellow color. This was taken as a starting point. The amount of bromate was increased for each successive sample until the volume of bromate represented an excess considerably over the theoretical amount necessary for addition.

Procedure. Samples of 0.7 gram were added to 20 ml. of 10% sulfuric acid (saturated with potassium bromide) and 15 ml. of *n*-heptane in a glass-stoppered flask, a specified volume of 0.5 *N* potassium bromate was added, and the mixture was shaken for 2 minutes. Potassium iodide was then added, and the liberated iodine titrated with 0.1 *N* thiosulfate, using starch as an indicator. The bromine number was calculated (cg. per gram), and the results were tabulated.

The values on di-, tri-, and tetraisobutylene, shown in Table II, indicate that some reaction besides addition is taking place; otherwise a constant value should be expected after the theoretical amount of bromate has been added.

Effect of Amount of Bromate on Substitution. The determination of addition and substitution on di-, tri-, and tetraisobutylene was made (1) by considering the sample as an unknown and determining unsaturation by the authors' original method (*9*), (2) as in (1) except that the theoretical amount of bromate was added, and (3) as in (1) except that the theoretical amount of bromate plus 1-ml. excess was added.

Procedure and calculations. Exactly 20 ml. of 10% sulfuric acid (saturated with potassium bromide) must be added to each sample in order to determine the increase of acidity due to formation of hydrobromic acid. A blank on the 10% sulfuric acid is first run by diluting the acid to 100 ml. in a glass-stoppered volumetric flask. A 5-ml. aliquot is pipetted into an Erlenmeyer flask containing 50 ml. of distilled water, and 10 ml. of a saturated potassium iodate solution and 2 ml. of saturated potassium iodide solution are added. The iodine liberated is titrated with 0.1 *N* sodium thiosulfate, and the equivalent acid is calculated as bromine.

Table II. Bromate-Bromide Method Showing Increase in Bromine Number with Increase of 0.5 *N* Bromate Solution

0.5 *N* $KBrO_3$, Ml./g.	(Theoretical Bromine No., Cg./Gram) 142.4 Diisobutylene	94.4 Triisobutylene	71.2 Tetraisobutylene
5	.....	.....	23.0
10	.....	.....	40.0
11	.....	44.0	....
15	.....	60.0	57.2
20	80.0	75.5	70.3
25	100.0	87.0	80.3
30	120.0	95.0	87.0
35	139.0	100.5	89.8
40	147.7	104.0	90.2
45	149.0	.....	....
50	150.5	.....	....

The apparent bromine number as represented by the amount of potassium bromate added is calculated in the usual manner:

$$\frac{0.08 \times \text{normality of } KBrO_3 \times \text{net ml. of } KBrO_3}{(\text{specific gravity} \times \text{volume of sample})} \times 100$$

The bromine equivalent of the 20 ml. of 10% sulfuric acid saturated with potassium bromide must now be calculated:

$$\frac{H_2SO_4}{2} \Rightarrow \frac{Br_2}{2} \Rightarrow \frac{I_2}{2} \Rightarrow \frac{Na_2S_2O_3}{1} \Rightarrow \frac{KBrO_3}{6}$$

Therefore 0.08 × normality of $Na_2S_2O_3$ × ml. of $Na_2S_2O_3$ × dilution factor = total grams of $Br_2 \Rightarrow$ 20 ml. of 10% H_2SO_4 (A)

In the determination of the apparent bromine number, the addition of bromate requires acid to liberate bromine according to the equation

$$3H_2SO_4 + KBrO_3 + 5KBr \rightarrow 3K_2SO_4 + 3Br_2 + 3H_2O$$

so that the amount of acid used to react with the potassium bromate in terms of bromine will be:

0.08 × normality of $KBrO_3$ × ml. of $KBrO_3$ = grams of Br_2 equivalent to H_2SO_4 used (B)

Thus the amount of sulfuric acid present at the start of the determination in terms of an equivalent amount of bromine is A−B, or C.

After the specified 2-minute shaking period and titration of excess bromate, the sample is treated in the following manner: The contents of the flask are transferred to a 250-ml. separatory funnel and washed thoroughly with water. The water layer is drained off as quickly as possible into a 100-ml. glass-stoppered volumetric flask, the solvent layer is washed several times with distilled water, and these washings are added to the flask. The solution is made up to volume, 5 ml. are transferred to an Erlenmeyer flask containing 50 ml. of distilled water, 10 ml. of a saturated potassium iodate solution are added, and the iodine is titrated with 0.1 *N* sodium thiosulfate. (The addition of potassium iodide at this point is not necessary, since it was added

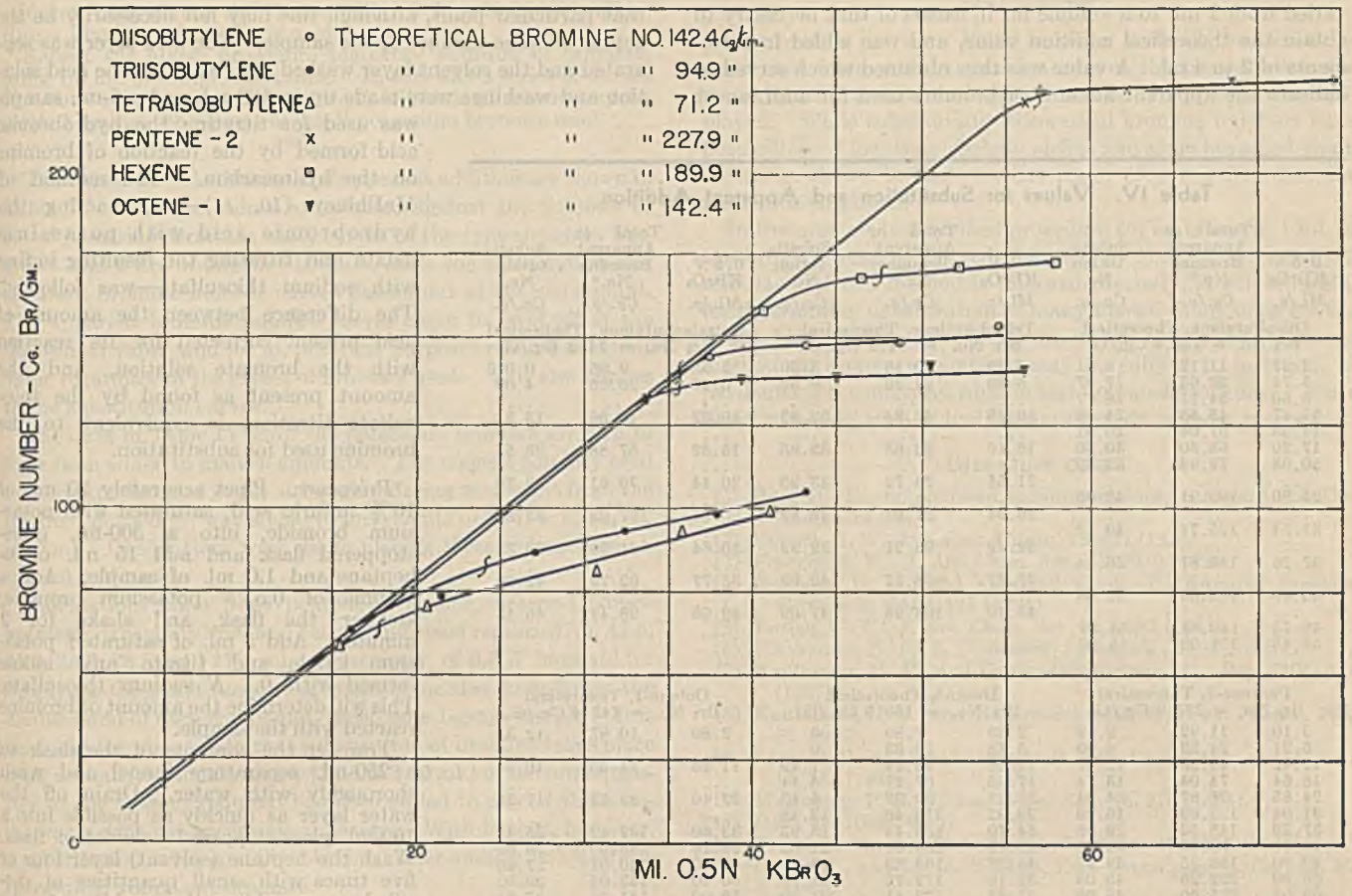

Figure 1. Total Bromine Number of Unsaturates
f = end point

earlier in the determination.) The total amount of acid present at the end of the determination will be calculated as in the following equation.

Table III. Addition and Substitution Using Bromate-Bromide Method with and without Modifications

	Theoretical Br_2 No.	Apparent Br_2 No.	Substitution No.	Bromine Consumed during Substitution Reaction	Addition No.
	Cg./g.	Cg./g.	Cg./g.	Cg./g.	Cg./g.
Diisobutylene, regular method (1 ml. excess $KBrO_3$)	142.4	144.0 143.6	57.6 56.4	115.2 112.8	28.8 30.8
Theoretical amount of $KBrO_3$ + 1-ml. excess		144.1 144.1	47.7 53.3	95.4 106.6	48.7 37.5
Theoretical amount of $KBrO_3$, no excess		141.8 141.8	55.4 54.3	110.8 108.6	31.0 33.2
Triisobutylene, regular method (1 ml. excess $KBrO_3$)	94.9	73.7 73.7	35.7 34.7	71.4 69.4	2.3 4.3
Theoretical amount of $KBrO_3$ + 1-ml. excess		86.6 85.9	40.6 41.6	81.2 83.2	5.4 2.7
Theoretical amount of $KBrO_3$, no excess		80.4 80.4	38.1 34.9	76.2 69.8	4.2 10.6
Tetraisobutylene, regular method (1 ml. excess $KBrO_3$)	71.2	39.9 45.5	19.3 21.4	38.6 42.8	1.3 2.7
Theoretical amount of $KBrO_3$ + 1-ml. excess		62.9 67.8	28.4 30.4	56.8 60.8	6.1 7.0
Theoretical amount of $KBrO_3$, no excess		58.7 63.2	28.0 30.0	56.0 60.0	2.7 3.2

0.08 × normality of $Na_2S_2O_3$ × ml. of $Na_2S_2O_3$ × dilution factor = grams of Br_2 equivalent to H_2SO_4 + HBr (D)

The difference, D − C, is the amount of bromine equivalent to the hydrobromic acid formed by substitution.

$$\frac{(D - C)}{\text{weight of sample}} \times 100 = \text{amount of substitution}$$

(in grams of bromine per grams of sample) which took place upon addition of the stated amount of potassium bromate.

Treating the sample as an unknown is necessary in order to find out how much halogen is due to addition as well as substitution when the determination is run in the regular way—i.e., by using 1-ml. excess of potassium bromate. In the present experiments, where the samples were considered as unknown, bromate was added until a brownish yellow color resulted.

The data in Table III show that substitution takes place when the determination is carried out by the bromate-bromide method in the regular manner, and also when this method is modified to the extent of using only the theoretical amount of bromate or the theoretical amount of bromate plus 1-ml. excess.

DETERMINATION OF APPARENT ADDITION AND SUBSTITUTION NUMBERS ON BRANCHED AND STRAIGHT-CHAIN OLEFINS

It has been found that a bromine substitution number can be obtained with or without saturation of the double bond for a wide variety of olefins when using a modification of the bromate-bromide method. This is illustrated by work on di-, tri-, and tetraisobutylene, pentene-2, hexene, and octene-1.

Changes in the procedure required that all factors except the bromate solution be kept constant in order to follow the course of both addition and substitution. The amount of bromate

varied from 2 ml. to a volume far in excess of that necessary to obtain the theoretical addition value, and was added in increments of 2 to 4 ml. A value was thus obtained which served to indicate the apparent amount of bromine used for addition at that particular point, although this may not necessarily be the actual bromine number of the sample. The acid layer was separated and the solvent layer washed with water. The acid solution and washings were made up to 100 ml. and a 5-ml. sample was used for titrating the hydrobromic acid formed by the reaction of bromine on the hydrocarbon. The method of McIlhiney (*10, 11*)—i.e., reacting the hydrobromic acid with potassium iodate and titrating the resulting iodine with sodium thiosulfate—was followed. The difference between the amount of acid present corrected for its reaction with the bromate solution, and the amount present as found by the thiosulfate titration, is equivalent to the bromine used for substitution.

Procedure. Pipet accurately 20 ml. of 10% sulfuric acid, saturated with potassium bromide, into a 300-ml. glass-stoppered flask and add 15 ml. of *n*-heptane and 1.0 ml. of sample. Add a volume of 0.5 *N* potassium bromate, stopper the flask, and shake for 2 minutes. Add 5 ml. of saturated potassium iodide and titrate any iodine formed with 0.1 *N* sodium thiosulfate. This will determine the amount of bromine reacted with the sample.

Transfer the contents of the flask to a 250-ml. separatory funnel and wash thoroughly with water. Drain off the water layer as quickly as possible into a 100-ml. glass-stoppered volumetric flask. Wash the heptane (solvent) layer four or five times with small quantities of distilled water, adding the washings to the volumetric flask. Make up to volume and transfer 5 ml. to an Erlenmeyer flask containing 50 ml. of distilled water. Add 10 ml. of a saturated potassium iodate

Table IV. Values for Substitution and Apparent Addition

0.5 *N* $KBrO_3$ *Ml./g.*	Total or Apparent Bromine No.[a] *Cg./g.*	Substitution No. *Cg./g.*	0.5 *N* $KBrO_3$ *Ml./g.*	Total or Apparent Bromine No.[a] *Cg./g.*	Substitution No. *Cg./g.*	0.5 *N* $KBrO_3$ *Ml./g.*	Total or Apparent Bromine No.[a] *Cg./g.*	Substitution No. *Cg./g.*
Diisobutylene, Theoretical Br_2 No. = 142.4 Cg./G.			Triisobutylene, Theoretical Br_2 No. = 94.9 Cg./G.			Tetraisobutylene, Theoretical Br_2 No. = 71.2 Cg./G.		
2.87	11.12	8.11	2.69	10.46	3.30	2.55	9.96	0.013
5.74	22.65	17.07	5.39	21.29	9.82	5.11	20.25	4.09
8.60	34.11	18.77	...	...	...	...	...	...
11.47	45.58	24.66	10.78	42.84	22.83	10.22	40.56	13.31
14.33	57.04	20.51	...	...	...	...	...	...
17.20	68.50	30.86	16.16	63.63	32.65	15.32	57.88	23.55
20.08	79.98	32.29	...	...	...	...	...	...
...	...	...	21.55	73.72	33.95	20.44	70.61	29.70
25.80	102.91	42.95	...	...	...	...	...	...
...	...	...	26.94	87.01	45.89	25.54	77.22	33.81
31.54	125.74	49.12	...	...	...	...	...	...
...	...	...	32.32	93.21	39.99	30.64	81.99	33.33
37.26	146.87	56.44	...	...	...	...	...	...
...	...	...	37.72	98.22	42.93	35.77	92.79	42.29
43.00	148.69	62.64	...	...	...	...	...	...
...	...	...	43.10	104.95	47.09	40.90	98.47	46.13
48.73	149.58	54.89	...	...	...	...	...	...
54.45	154.69	58.30	...	...	...	...	...	...
Pentene-2, Theoretical Br_2 No. = 227.9 Cg./G.			Hexene, Theoretical Br_2 No. = 189.9 Cg./G.			Octene-1, Theoretical Br_2 No. = 142.4 Cg./G.		
3.10	11.92	2.49	2.89	7.89	0	2.80	10.87	12.31
6.21	24.32	4.99	5.78	20.33	0	...	...	...
12.42	49.23	12.47	11.56	43.24	1.49	11.20	44.55	10.34
18.64	74.04	13.74	17.35	67.17	5.11	...	...	...
24.85	98.87	24.94	23.12	90.32	6.42	22.40	89.33	17.36
31.04	123.69	16.28	28.92	113.46	13.48	...	...	...
37.29	148.52	29.96	34.70	136.43	15.95	33.60	132.49	25.47
43.50	173.35	32.38	40.48	159.81	21.86	39.20	139.00	28.69
49.70	198.35	39.97	46.26	168.82	18.29	44.80	140.01	27.46
55.90	222.26	45.08	52.10	172.72	21.05	50.40	143.05	29.02
62.08	225.90	48.96	57.85	173.41	21.50	56.00	141.22	27.65
68.30	228.11	58.98	...	...	...	...	...	...
74.55	228.35	57.64	...	...	...	...	...	...

[a] Apparent addition at any given point.

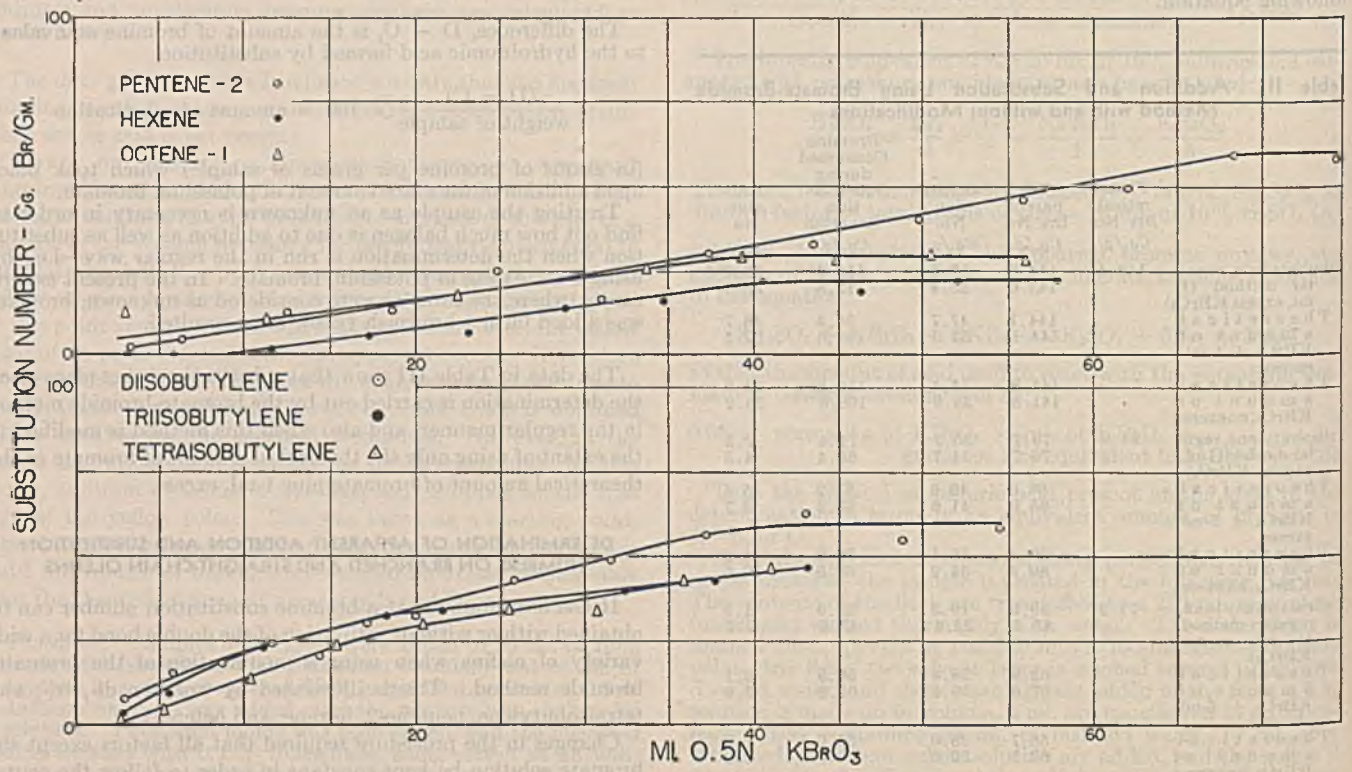

Figure 2. Substitution in Unsaturates

solution, and titrate the iodine with 0.1 *N* sodium thiosulfate. Run a blank on the sulfuric acid.

Repeat the above procedure for every addition of bromate, using 1.0 ml. of sample each time. In this way a series of values (for both substitution and addition) is obtained which may be plotted against the volume of 0.5 *N* potassium bromate used.

The values for substitution and apparent addition are shown in Table IV. If these values are plotted against the volumes of 0.5 *N* potassium bromate used, curves of the type shown in Figures 1 and 2 are obtained. In most cases the substitution and apparent bromine number curves flatten out at the same point. The apparent bromine number curves begin to level off at the theoretical value, and for all practical purposes reach a constant value regardless of the excess of bromate used. This also applies to the substitution curves.

The data in Table IV show the potassium bromate solution to have been added in uneven amounts. The reagent actually used for the experimental work, although differing somewhat from the required normality, was added in increments of 2, 4, 6, 8, 10, 12, etc., ml. Calculations were made placing these volumes on an exact 0.5 *N* basis.

Calculations show that pentene-2, hexene, octene-1, diisobutylene, triisobutylene, and tetraisobutylene require 57.0, 47.5, 35.6, 35.6, 23.8, and 17.8 ml., respectively, of 0.5 *N* bromate for supplying the exact amount of bromine needed to saturate the double bond of each compound, based on a 1-gram sample. It is shown in the above data that substitution of bromine takes place after a small fractional volume (2.55 to 3.10 ml.) of the total quantity of the reagent required has been added to any of these unsaturates with the exception of hexene. With hexene, however, a substitution value could be obtained after adding 24.3% of the theoretical volume of bromate.

CONCLUSIONS

Substitution has been found to occur with all the olefins investigated when various modifications of the bromate-bromide method, using potassium bromide–saturated reagents, were employed. While substantially theoretical bromine numbers have been obtained for straight-chain olefins and some branched-chain olefins as shown in the previous paper (*9*), it is felt that the method is empirical.

In the previously described procedure (*9*) an excess of 1 ml. of the bromate reagent is used, followed by shaking for exactly 2 minutes. If this method is followed precisely, it will be useful for determining unsaturation of many known compounds having double bonds. However, bromine numbers obtained by the same method on unknown mixtures may be subject to question, in particular if the mixtures contain highly branched olefins. More direct methods, such as hydrogenation, may be more accurate.

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Absorption Spectra of Volatile Essential Oils

Detection of Alpha-Dicarbonyl Compounds

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A test devised for detection of α-dicarbonyl compounds in fatty materials has been extended to volatile essential oils. Tests on juniper berry, orange peel, and coriander seed oils are reported, as evidence that the flavor value of botanicals may be rapidly and accurately estimated by determination of the extinction values or α-dicarbonyl content of volatile essential oils used in the manufacture of alcoholic beverages.

THE quality of alcoholic beverages such as gin, cordials, and liqueurs can be maintained only by rigid chemical control and taste tests. Flavor control starts with the correct selection of botanicals and other materials used for their flavor value. Specifications used for the purchase of botanicals are chemical constants, necessitating long chemical determinations, but the final decision rests upon whether the product, made either in the factory or in the laboratory, meets the flavor approval of a quality committee (*5*). Some rapid method such as the determination of the extinction values or the α-dicarbonyl content of the volatile essential oil might be correlated with the flavor examination of the finished product, thereby giving a rapid and accurate method for the estimation of the flavor value of the botanical.

At the present time extinction values and α-dicarbonyl values are included in the specifications for the purchase of raw materials, for it is believed that they give some indication of the geographical growing district and the age of the botanical. Both the growing district and the age are very important from a flavor standpoint.

Volatile essential oils were obtained from botanicals, many of which were purchased during 1939 and had been in cold storage for periods varying from a few to 30 months. The essential oils were not commercially rectified but were recovered by the Clevenger method of steam-distillation (*3*). If the distillation is continued for at least 8 hours, the volatile essential oils obtained frequently have a yellow color, especially if the oil has been distilled from botanicals that have been in storage for some time. The color might be caused by α-dicarbonyl compounds which impart color to autoxidized oils of animal and vegetable origin (*4*). The exact mechanism leading to the formation of colored substances is not fully understood, but there is agreement on the assumption that the yellowing is caused by diketo groups when fatty oils age. A simple test devised for the detection of α-dicarbonyl compounds in fatty materials (*4*) has been extended to volatile essential oils.

EXPERIMENTAL

Methods. Physicochemical data on the oils, such as index of refraction, specific gravity, acid number, and ester number, were obtained by standard procedures (*2*).

[1] Present address, Horlick's Malted Milk Corporation, Racine, Wis.

Transmittance measurements of the oils were made with a Coleman Universal spectrophotometer. The measurements would have been of greater significance in the ultraviolet range of the spectrum in which maxima and minima would have been more clearly defined. The undiluted oils were placed directly in transmission cells of 1-cm. diameter, and compared with a blank cell containing the same quantity of water. Extinction values (log $1/T$) were read off directly from the drum scale of the instrument.

REAGENTS. Oximation solution, 3% hydroxylamine hydrochloride in pyridine. Solutions for forming the bis-pyridine-ferrous derivatives of dioximes: 60 grams of Rochelle salt plus 100 ml. of water; 5 grams of ferrous sulfate plus 100 ml. of water; 100 grams of potassium hydroxide plus 100 ml. of water.

METHOD. Pipet 0.5 gram of volatile oil into a test tube and oximate by adding 2 ml. of hydroxylamine hydrochloride solution. React for 2 hours in a water bath at 80° C.; cool, add 0.3 ml. of acetone to destroy the excess of hydroxylamine, and let stand for 5 minutes. The highly colored bis-pyridine-ferrous derivative of the dioxime is formed as follows: Add 1 ml. of Rochelle salt solution and 1 ml. of ferrous sulfate solution, then slowly add, with shaking, 4 ml. of potassium hydroxide solution. A positive test for α-dicarbonyl compounds is the formation of an intense red color in the pyridine layer. A blank is run with reagents only.

The test was run on solutions containing known amounts of biacetyl and dimethylglyoxime; the preliminary oximation procedure is not necessary for the dimethylglyoxime. Solutions were made by weighing out 10, 20, 30, 40, and 50 millimoles, dissolving in alcohol, and diluting to 1 liter. The colors developed by the oils were visually compared with the colors developed by the known solutions. In this way a semiquantitative estimation of millimoles of α-dicarbonyl compounds present in the original volatile oils could be made.

JUNIPER BERRY OIL

Extinction values were obtained using fifteen oils, steam-distilled from shipments of juniper berries, *Juniperus communis*, some of which had been used in the plant production of gin. Therefore the flavor values of some of the berries have been evaluated by actual use in production.

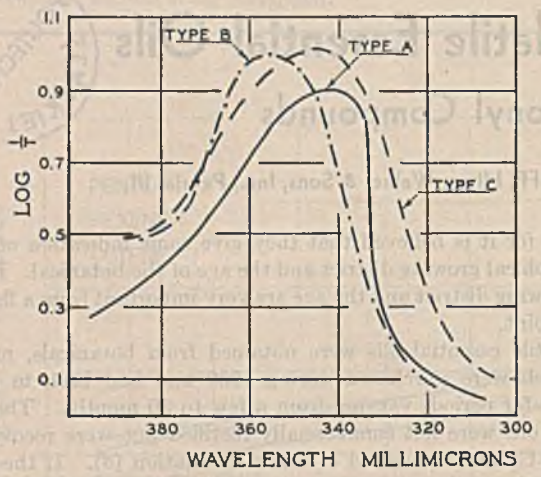

Figure 1. Absorption Curves of Juniper Berry Oil Types

Type A, oil of ordinary Italian berries
Type B, oil of select Italian berries
Type C, oil of domestic berries

Figure 1 shows that juniper berry oils obtained from berries which had been in cold storage for 25 to 30 months give three distinct curves.

Type A with a maximum extinction value at 340 mμ represents oils distilled from ordinary Italian berries scooped from the ground and bushes; type B with a maximum extinction at 355 to 360 mμ represents oils distilled from berries which were hand-picked and selected in Italy; type C with a maximum extinction at 345 mμ represents oil distilled from berries grown in eastern United States. The berries which produced the finest flavored gins when used in the factory contained oils which gave a maximum extinction at 355 to 360 mμ, such curves rising rapidly and falling almost as soon as the maximum extinction is reached; the whole shape of the curve is almost as important as the maximum extinction value. Berries whose oils give extinction values similar to type A are inferior in quality, and berries whose oils give extinction values similar to type C are very much inferior from a flavor standpoint. Therefore, the extinction values of an oil distilled from an unidentified source of juniper berries will give information as to the flavor value of such berries.

A commercial juniper oil, Juniperol (Fritzsche Bros.), distilled between 160° and 200° C. in the laboratory gave a curve much like type A oils except the maximum extinction value was 0.7 instead of 0.9.

Table I. Aging of Juniper Berry Oils within the Berry and in Soft-Glass Bottles

Oil No.	Description	Extinction Values at Following Wave Lengths 320 mμ	340 mμ	350 mμ	360 mμ	370 mμ	380 mμ
1	Freshly distilled from berries	0.18	0.90	1.20	1.10	0.92	0.74
2	Oil 1 aged 60 days in soft glass	0.20	0.94	1.18	1.20	1.05	0.72
3	Freshly distilled from berries stored in cold for 30 months	0.18	1.06	1.05	0.85	0.67	0.54
4	Freshly distilled from same berries stored in cold for 46 months	0.60	1.10	1.22	1.22	1.00	0.84

Most of the volatile oils deteriorate rapidly unless kept in small, filled, tightly stoppered bottles in a cool, dark place. If exposed to air, light, and warmth they resinify, and the oxidized resinous products formed change the volatile oils in composition, color, specific gravity, and odor. There is spectroscopic evidence that aging does occur in the botanical during cold storage for 25 to 30 months, but at a much less rapid rate than during an additional 30 months' storage. Aging in the botanical during cold storage for the next 15 months after 30 months' storage is very rapid and almost equal to aging of the oil outside the botanical.

The steam-distilled juniper berry oils were stored in soft-glass bottles for 60 days at room temperature on top of the laboratory table away from the sunlight. An example of the change due to such accelerated aging can be demonstrated by spectral data.

Oil 1, Table I, shows the extinction values for freshly distilled juniper oil from the berries, and oil 2 shows the extinction values for the same oil after accelerated aging. Aging of the juniper oils in every case shifts the extinction maximum toward the light of higher wave lengths, and the near maximum is maintained over a greater wave-length span. The extinction values in the higher wave lengths do not fall as rapidly as those of the fresh oil. Some of the juniper oils show more resistance to accelerated aging than others, and in most cases the extinction values increase greatly in the higher wave lengths. Oils stored in the dark at icebox temperatures show no change in the spectral data.

Oil 3, Table I, shows the spectral extinction values of a freshly distilled juniper oil taken from berries which had been stored at refrigerator temperatures for 30 months. Oil 4, Table I, shows the spectral extinction values of the freshly distilled oil taken from the same berries after cold storage for 46 months. There has been rapid aging of the oil in the berries between 30 and 46 months of storage. Extinction values will give some evidence of the aging of oil in the berries, and such data will make it possible to purchase fresh stocks of raw materials and maintain a high flavor level.

Table II shows the change in some of the chemical constants of the juniper oils after aging. The index of refraction, specific gravity, acid number, and ester number increase rapidly when the oil is stored in soft-glass bottles placed on the laboratory desk top at room temperature. Oil distilled from domestic juniper berries shows a great increase in density and ester number when aged outside the berry. Resinification was very noticeable when the domestic berry oil was aged outside the berry, but there was little change in the oils steam-distilled from the Italian berries.

Table II. Chemical Constants of Steam-Distilled Juniper Oils

(Before and after aging in soft-glass bottles at laboratory temperatures and room light)

Source of Oil	Index of Refraction, 20° C.	Specific Gravity, 25° C./25° C.	Acid No.	Ester No.
Ordinary eastern United States berries				
Fresh oil	1.4780	0.8530	1.05	8.98
Aged oil	1.4880	0.8815	1.91	11.77
Ordinary Italian berries				
Fresh oil	1.4800	0.8725	1.71	6.91
Aged oil	1.4830	0.8807	2.23	8.31
Italian hand-picked and selected berries				
Fresh oil	1.4812	0.8626	1.15	5.25
Aged oil	1.4825	0.8775	1.92	5.15

Table III. Chemical Constants and α-Dicarbonyl Content of Juniper Oils

Sample No.	$n_D^{20°}$	Acid No.	Ester No.	Millimoles of α-Dicarbonyl Compounds per Kg. of Volatile Oil
1	1.4782	0.98	3.4	0
2	1.4760	0.83	7.4	0
3	1.4811	..	..	0
4	1.4788	1.07	8.1	20
5	1.4788	1.07	6.8	20
6	1.4792	1.07	6.3	30
7	1.4812	2.50	9.7	50
8	1.4828	2.90	11.9	50
9	1.4790	9.10	18.1	90
10	1.4802	7.00	20.6	90

All the aged oils had a turpentinelike odor. The juniper berry oil aged in the berry and stored in cold storage between 30 and 46 months showed increases in acid and ester number.

Table III shows the chemical constants and α-dicarbonyl content of various steam-distilled juniper berry oils.

Oils 1, 2, and 3 were distilled from hand-picked and selected Italian juniper berries stored for 30 months in cold storage. No α-dicarbonyl compounds could be detected in these volatile oils. Oils 4, 5, and 6 were distilled from Italian berries stored for 46 months in cold storage. Oils 7 to 11 were distilled from juniper berries that had been stored in sealed tin containers at room temperature for 5 years, berries 7 and 8 were select Italian berries, and berries 9 and 10 were ordinary German juniper berries. The increase in α-dicarbonyl content is in direct correlation with the age of the berries.

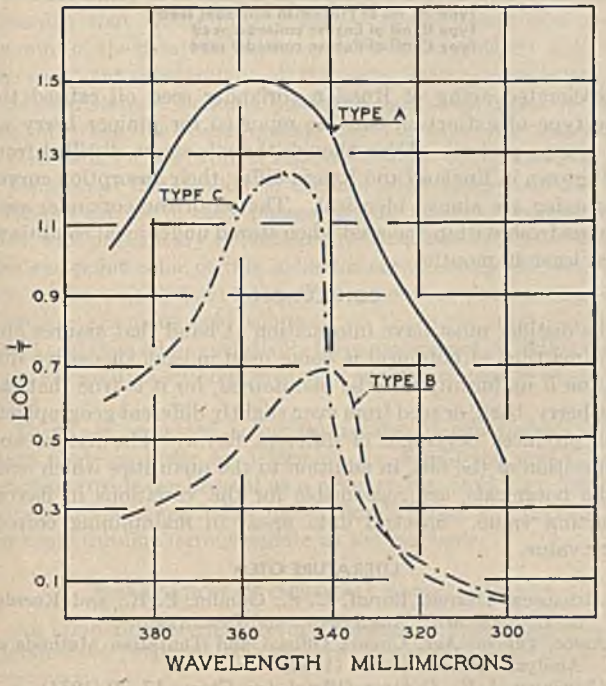

Figure 2. Absorption Curves of Orange Peel Oil Types

Type A, oil of bitter orange peel
Type B, oil of Florida ribbon orange peel
Type C, oil of Sicilian sweet orange peel quarters

Unsaturated carbonyl compounds show bands of strong absorption at low wave lengths, but absorption in the higher wave lengths, such as used for the experimental work of this paper, are said to be due to conjugated systems. The intensive absorption bands are displaced to longer wave lengths with increasing length of the chromophoric conjugated systems. The atmospheric distillation of aged commercial juniper oil showed that the compounds responsible for the shift in maximum extinction value could be concentrated in the residue. Treatment of the aged commercial juniper oil with activated carbon changed the extinction values slightly, but it was not possible to rejuvenate the aged oil by carbon treatment. Acetylation of a very pure fraction of commercial juniper oil moved the extinction maximum from 340 to 370 mμ and gave values similar to aged juniper oil.

Table IV shows the α-dicarbonyl content, acetyl number, and months in cold storage for a number of juniper oils. The acetyl number can be used as a method of differentiation between domestic and select Italian berries. There was definite positive correlation between the α-dicarbonyl content and the acetyl number of the juniper oils.

Table IV. Comparison of α-Dicarbonyl Content and Acetyl Number of Freshly Distilled Juniper Oils

Identification	Months in Cold Storage	Relative α-Dicarbonyl Content	Acetyl No.
Select Italian	46	2	41.9
Select Italian	46	1	45.3
Select Italian	45	3	43.6
Ordinary Italian	46	3	70.4
Ordinary Italian	46	3	76.8
Select Italian	42	2	52.3
Select Italian	39	0	35.2
Ordinary United States	17	3	106.0

ORANGE PEEL OIL

Orange peel, bitter and sweet, is used in the manufacture of cordials and liqueurs. Bitter orange oil differs very slightly from sweet orange oil physically and chemically; hence specifications for the purchase of orange peel do not always help to select the peel with the correct flavor character. Extinction values and the odor of the acetylated oil freshly distilled from an unknown peel will be helpful in identifying true bitter orange peel.

Extinction values were obtained using 15 orange oils steam-distilled from shipments of bitter peel, imported sweet quarter peel, and ribbon peel of sweet oranges grown in the United States. Any single oil examined gave one of the three types of absorption curves shown by Figure 2. Oils steam-distilled from bitter peel showed maximum extinction at 355 mμ (type A), oils steam-distilled from domestic sweet orange ribbon and Haitian ribbon peel showed maximum extinction at 340 mμ (type B), and oils steam-distilled from Sicilian sweet orange peel quarters showed maximum extinction at 350 mμ (curve C). Practical plant use of the many sweet orange peels used in this study demonstrated that Florida sweet ribbon peel can be used in place of Haitian ribbon peel for sweet orange distillates; both peels have the same spectral identity.

The steam-distilled orange oils were stored in soft-glass bottles, partially filled, on the laboratory table at room temperature. The oils were very prone to oxidation, and quickly deteriorated in flavor value during such storage conditions. Table V, oil 1, shows the extinction values of a fresh oil distilled from Florida ribbon peel; oil 2 shows the extinction values for the same oil after accelerated aging for 60 days. Aging of the oil shifts the extinction maximum towards the higher wave lengths and greatly increases the optical density at its maximum (1.5 instead of 0.5 extinction).

Orange oils freshly distilled by the Clevenger method, ir-

Table V. Aging of Sweet Orange Peel Oil and Effect of Adding Ethyl Anthranilate and Decyl Aldehyde to Sweet Orange Oil

Oil No.	Description	Extinction Values at Following Wave Lengths 320 mμ	340 mμ	350 mμ	360 mμ	370 mμ	380 mμ
1	Freshly distilled from Florida ribbon peel	0.10	0.52	0.46	0.35	0.31	0.26
2	Oil 1 aged 60 days in soft glass	0.10	1.03	1.25	1.44	1.50	1.43
3	Commercial sweet orange oil, fraction distilled at 174–177° C.	0.20	0.58	0.58	0.50	0.37	0.28
4	Addition of ethyl anthranilate to oil 3	0.30	0.90	1.13	1.27	1.12	0.90
5	Addition of decyl aldehyde to oil 3	0.20	0.58	0.58	0.50	0.39	0.31

Table VI. Chemical Constants of Steam-Distilled Sweet Orange Oils

(Before and after aging in soft-glass bottles at laboratory temperatures and room light)

Source of Oil	Specific Gravity, 25° C./25° C.	Ester No.	Acid No.
Florida ribbon peel			
Fresh oil	0.8403	2.30	0.7
Aged oil	0.9242	44.62	5.5
Sicilian peel in quarters			
Fresh oil	0.8420	4.30	0.8
Aged oil	0.9342	51.30	8.7

respective of the age of the peel or the storage conditions, gave negative tests for α-dicarbonyl compounds. Even after the accelerated aging period of 60 days many of the oils contained less than 10 millimoles of α-dicarbonyl compounds per kg. of oil. It was noticed that oils distilled from peels with a very small amount of pulp developed a higher α-dicarbonyl content during the aging period of 60 days. Perhaps there is an antioxidant in the pulp of the peel.

Table VI, chemical constants of fresh and aged sweet orange oils, shows a great increase in ester number for each aged oil.

In order to understand better what chemical change is responsible for the shift in extinction maxima of sweet orange oils during aging, ethyl anthranilate (Felton Chemical Co.) and decyl aldehyde (Florasynth Laboratories), both freshly distilled at atmospheric pressure, were added to the fraction (b.p. 174–177° C.) of oil of sweet orange (Fritzsche Brothers, U.S.P.) whose extinction values are shown as oil 3, Table V. When 0.5 ml. of ethyl anthranilate was added to 10 ml. of oil 3, the extinction maximum shifted towards the visible light, oil 4, Table V. When 0.1 ml. of decyl aldehyde was added to 10 ml. of oil 3, the extinction values remained unchanged (oil 5, Table V). None of the individual ester, aldehyde, or oil had a visual yellow color.

Atmospheric distillation of commercial bitter orange oil into two fractions and residue showed that the compounds responsible for the extinction maximum shift of aged oil can be concentrated in the residue just as was done with juniper oil. The extinction values of the first fraction (b.p. 175–177° C.) were identical to those of a good sweet orange oil. The second fraction (b.p. 177–185° C.) gave extinction values characteristic of good bitter orange oil. The spectral data that enable one to differentiate between bitter and sweet oil and thereby ensure good flavor are due to fraction 2 and the residue. Over one half of the bitter orange oil is the same chemically when measured by spectral data as sweet orange oil.

Acetylation of all the fractions of bitter orange oil caused the formation of a yellow color or intensification of the yellow color. During the course of acetylation it was very noticeable that all fractions of acetylated bitter orange oil had a foreign, characteristic odor. Fraction 1 had an acetyl number of 11.9, fraction 2 had an acetyl number of 21.4, and the residue had an acetyl number of 50.1. The characteristic odor formed by acetylation will give an additional test to differentiate bitter orange oil from sweet orange oil, thereby maintaining the correct balance of flavor in the manufacture of cordials and liqueurs.

The acetyl numbers of sweet orange oils steam-distilled from orange peels which have been stored for about 40 months were lower than fraction 1 of the bitter orange oil. This is additional evidence of antioxidants in the peel.

CORIANDER SEED OIL

The fruit of the *Coriandrum sativum*, known as coriander seed, is used as an ingredient in the manufacture of gin. Seeds have been offered for sale from at least nine geographical growing districts of Europe and Africa. Ultraviolet absorption studies have been made of the alcoholic solutions of the various essential oils obtained by the Clevenger steam-distillation of the seeds. The extinction values showed that the distilled oils from seeds grown in different geographical districts could be differentiated from one another by their absorption curves (*1*).

Extinction values were obtained using 5 coriander oils distilled from shipments designated as seeds grown in England, Hungary, and Russia. The absorption curves for Hungarian seed oil (type A), English seed oil (type B), and Russian seed oil (type C), all of Figure 3, show a great likeness between the Russian and Hungarian oils; the English oil is different. Russian and Hungarian seeds are more desirable from a flavor standpoint for the manufacture of gin than are seeds of English origin.

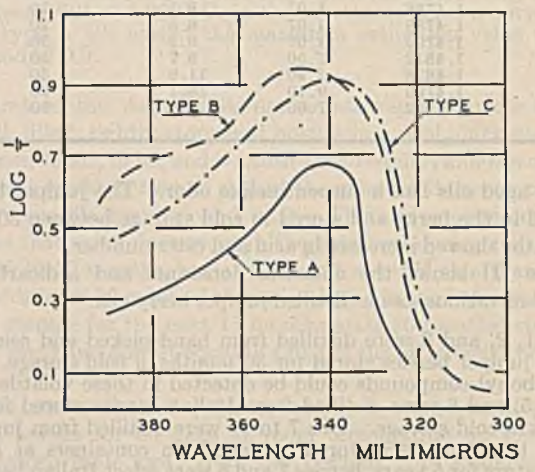

Figure 3. Absorption Curves of Coriander Seed Oil Types

Type A, oil of Hungarian coriander seed
Type B, oil of English coriander seed
Type C, oil of Russian coriander seed

Accelerated aging of Russian coriander seed oil caused the same type of extinction shift as reported for juniper berry oil and orange peel oil. Even though the oils steam-distilled from seeds grown in England and Russia differ, their absorption curves after aging are almost identical. The oil of the coriander seed remains fresh within the seed when stored under ideal conditions for at least 40 months.

CONCLUSION

The distiller must have information at hand that assures him the same type of botanical is being used in each successive distillation if uniformity is to be maintained, for it is true that the same berry, bark, or seed from even slightly different geographical areas produces beverages of different flavor. The nature and composition of the oils, in addition to the quantities which exist in the botanicals, are responsible for the variations in flavor-imparting value. Spectral data assist in maintaining correct flavor value.

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Use of Iodine Monochloride in Standardization of Permanganate Solutions with Arsenious Oxide

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Permanganate solutions can be standardized against arsenious oxide by using iodine monochloride as a catalyst and o-phenanthroline ferrous complex as indicator. The end point is attained rapidly, is stable, and no correction for the catalyst is required regardless of the amount added. Results obtained agree with values obtained with oxalate by the method of Fowler and Bright to within 1 part in 3000.

EXPERIMENTAL studies by Kolthoff, Laitinen, and Lingane (*5*) and Bright (*1*) have confirmed the observations made by Lang (*6*) and subsequently by others (*2, 7–9*) that arsenious oxide can be used as a satisfactory primary standard for permanganate solutions provided an iodine compound is added to the titrated solution. Kolthoff and co-workers (*5*) and Bright (*1*) made use of both potentiometric and visual end points; the visual end points were obtained both by the permanganate color and by using *o*-phenanthroline (1,10-phenanthroline)–ferrous sulfate complex as an internal potential indicator.

Potassium iodate was used by Kolthoff and his co-workers, and either potassium iodide or iodate by Bright; in all cases 1 drop of a 0.0025 formal[1] solution of the iodine compound was used in final volumes of from 120 to 175 ml. Upon making titrations under the conditions outlined by the above workers, and using visual end points, it was evident that the rate of the titration reaction became slow in its final stages. Even 2 ml. before the end point, when using approximately 0.02 formal (0.1 *N*) permanganate solutions, the pink color of the *o*-phenanthroline indicator faded upon addition of each drop of permanganate and 5 to 10 seconds were required for the original color to return; closer to the end point this effect became much more pronounced. As a result, considerable time and patience are required to establish the correct end point, and persons not familiar with the end point are likely to obtain results significantly in error. It seemed probable that this difficulty might be minimized if a larger amount of the catalytic iodine compound were added; also, if at the end point substantially all the iodine were present in a single oxidation state, it would be possible to add a compound in which the iodine had this oxidation state and thus avoid the necessity of a correction for the catalyst regardless of the amount added.

Because of the stability of the end point in hydrochloric acid solutions the use of the *o*-phenanthroline indicator seemed desirable, and preliminary potentiometric titrations indicated that at the end-point color of this indicator substantially all the iodine was in the unipositive state. This conclusion was confirmed by titrating arsenious acid solutions to the *o*-phenanthroline end point and then adding relatively large amounts of an iodine monochloride solution without causing a significant change in the end point.

There are presented below the results of experiments which were made in order to determine the conditions under which arsenious oxide can be used as a primary standard for permanganate solutions when iodine monochloride is used as a catalyst and *o*-phenanthroline ferrous sulfate as the indicator.

PREPARATION OF CHEMICALS AND SOLUTIONS

An approximately 0.02 formal solution of potassium permanganate was prepared and allowed to age for 2 months, then filtered through a glass filter, and stored in an all-glass light-protected bottle. A 0.002 formal solution of potassium permanganate was prepared daily by accurately diluting the 0.02 formal solution.

Bureau of Standards sodium oxalate No. 40c was used, and the purity taken as 99.95%. Bureau of Standards arsenious oxide No. 83 was also used, and the purity taken as 99.98%. Each was dried for 1 hour at 105° C. immediately before use.

An iodine monochloride solution was prepared by the reaction of potassium iodide and iodate in 4 formal hydrochloric acid, using the disappearance of the carbon tetrachloride–iodine color to determine the end point; it was standardized with sodium thiosulfate. A 0.0025 formal solution was prepared by diluting the above solution with 2 or 4 formal hydrochloric acid.

Six formal sodium hydroxide was prepared and stored in a glass bottle provided with a paraffin-coated glass stopper. Rubber-stoppered bottles were found unsatisfactory for the storage of sodium hydroxide solutions which are to be used in oxidimetry.

Approximately 0.05 formal sodium oxalate solutions were gravimetrically prepared in 1.8 formal sulfuric acid (*4*). These solutions were used for no longer than 2 days.

Approximately 0.05 formal arsenious acid solutions were prepared by dissolving the weighed arsenious oxide in 25 ml. of 6 formal sodium hydroxide, adding 63 ml. of 12 formal hydrochloric acid and 2.7 grams of sodium chloride, and diluting to 500 grams.

The potential indicator was 0.025 formal *o*-phenanthroline ferrous sulfate as obtained from the G. Frederick Smith Company.

PRELIMINARY EXPERIMENTS

A series of titrations was made using the *o*-phenanthroline indicator procedure of Bright (*3*) except that successively increasing volumes of the 0.0025 formal iodine monochloride solution were added. With 0.25 ml. of the catalyst, the pink color of the indicator faded temporarily to a perceptible violet color upon the addition of a drop of 0.02 formal permanganate approximately 0.2 ml. before the end point; with 0.5 and 1 ml. of catalyst only a transient fading of the pink color was observed; and with 5 ml. of catalyst fading was observed when about 0.1 ml. from the end point. The first end point (a very pale blue color) was transient, and the pink color returned more slowly, and an additional drop of permanganate (0.02 *F*) was required. The resulting end point was usually stable. The return of the end point with 5 ml. of catalyst is attributed to a slow rate of oxidation of elementary iodine by permanganate under the conditions of the titration.

The characteristics of the end point when using in one case 0.04 ml. of 0.0025 formal potassium iodate—the amount of catalyst used previously (*1, 5*)—and in the other case 1.0 ml. of 0.0025 formal iodine monochloride were compared by titrating 2-ml. portions of 0.075 formal arsenious acid. The behavior of the indicator with the successive additions of permanganate is shown in Table I.

Table I. Titration Characteristics with Varying Amounts of Iodine Catalyst

(Volume: approximately 100 ml. of solution 0.5 *F* in HCl and 0.5 *F* in NaCl. Indicator: 0.04 ml. of 0.025 *F* *o*-phenanthroline ferrous sulfate. Catalyst: 0.04 ml. of 0.0025 *F* KIO_3 or 1 ml. of 0.0025 *F* ICl)

$KMnO_4$ Added, Ml.		Observations	
Increment	Total	KIO_3 catalyst	ICl catalyst
0.04	0.04	Solution almost colorless	Pink faded slightly
0.08	0.12	Transient end point	Pink faded slightly
0.50	1.00	End point for 2 seconds	Pink faded slightly
0.50	1.50	End point for 2 seconds	End point for 0.5 second
0.50	2.00	End point for 3 seconds	End point for 0.5 second
0.25	2.75	End point for 3 seconds	End point for 0.5 second
0.04	3.14	End point for 1 second	Pink color faded slightly
0.04	3.18	End point for 4 seconds	Pink color faded slightly
0.04	3.22	End point for 13 seconds	End point for 1 second
0.03	3.25	End points permanent	

[1] Formal concentrations, formula weights per liter of solution, have been used because of the uncertainty attached to the use of normal concentrations with such compounds as potassium iodate and iodine monochloride which may have various changes of oxidation state.

Table II. Standardization of a Permanganate Solution against Sodium Oxalate and Arsenious Oxide

(Two separate solutions of both oxalate and arsenious oxide were prepared and series of titrations made using weighted portions of each solution. The values shown are the weight normalities of the permanganate.)

	Using Sodium Oxalate		Using Arsenious Oxide	
	Solution A	Solution B	Solution C	Solution D
	0.10792	0.10788	0.10791	0.10793
	0.10789	0.10788	0.10793	0.10791
	0.10793	0.10790	0.10794	0.10794
	0.10792	0.10793	0.10794	.....
	0.10787	0.10791	.....	.....
Av.	0.10791	0.10790	0.10793	0.10793
Average deviation, %	0.019	0.015	0.009	0.009
Maximum deviation, %	0.06	0.05	0.03	0.03

A series of comparison titrations was then made, in order to check the precision of titrations made with the large amount of iodine monochloride and the agreement which could be obtained with the titer values obtained using sodium oxalate.

TITRATION PROCEDURES

Titration of Arsenious Acid Solution with Permanganate. Approximately 50 grams of the arsenious acid solution were weighed out into a 400-ml. beaker and diluted to 100 ml. with water (giving a solution 0.2 formal in sodium chloride and 0.6 formal in hydrochloric acid). One milliliter of 0.0025 formal iodine monochloride in 4 formal hydrochloric acid was added and the 0.02 formal potassium permanganate solution was added rapidly from a weight buret, with continuous stirring, until a lag in the decolorization of the permanganate was noticed. The permanganate was then added dropwise (still with continuous stirring) until the permanganate color spread almost throughout the solution. (The indicator is advantageously withheld until this preliminary indication is obtained, since it permits rapid addition of permanganate; otherwise the pink color of the phenanthroline complex would prevent observation of the permanganate color.) There was then added 0.04 ml. of the indicator and the titration was continued dropwise with the 0.02 formal permanganate until the first transient fading of the indicator was detected; this is usually within 1 drop of the end point. The 0.002 formal permanganate was then added from a 10-ml. volume buret until the pink color could no longer be detected. A blank solution was prepared, the end point checked again, and the blank solution titrated. The blanks required from 0.15 to 0.20 ml. of the 0.002 formal permanganate.

Titration of Sodium Oxalate with Permanganate. The procedure of Fowler and Bright (*3*) was followed in detail.

The results of the titrations are shown in Table II.

ACKNOWLEDGMENT

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Determination of 2,3-Butylene Glycol in Fermentations

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Brockmann and Werkman (*1*) described a method for the determination of 2,3-butylene glycol in fermentations, which was much more satisfactory than previous methods, but had a number of defects. Recoveries were low, averaging 96.4%. The distillation step, involving collection of 1 liter of distillate from 25 ml. of solution, was cumbersome. Moreover, if sugar was present in the sample, interfering substances were formed during the alkaline distillation. The sample required was large (20 mg. or more).

In the procedure described below, the butylene glycol is separated from the culture by continuous ether extraction. Periodate oxidation is used, as in the method of Brockmann and Werkman. The acetaldehyde is determined by titration of the sulfite liberated when the acetaldehyde-bisulfite complex is made alkaline, in a step practically identical with that used by Friedemann and Graeser (*2*) in lactic acid determination. Iodometric acetaldehyde titration has been applied to butylene glycol determination in urine by Westerfeld and Berg (*5*). Recoveries are high (99%) and reproducible. There is little interference from constituents of culture media. From 2 to 10 mg. of butylene glycol are required.

This procedure has been in use for more than 5 years, and has given reliable results in student laboratories.

EXTRACTION

Since 2,3-butylene glycol is extracted relatively slowly from water by ether, continuous extraction is necessary for from 8 to 48 hours, depending on the design of the extraction apparatus. In the author's laboratory, small continuous extractors, holding 5 ml. of sample, are used in routine determinations. A battery of these, attached to a multiple condenser and heated by one steam plate, are used to extract for 12 hours or more 5 ml. of culture or diluted culture, adjusted to pH 7 or higher (to prevent extraction of lactic acid, which interferes). The time necessary for complete extraction should be determined by experiment before a standard extraction time is selected. Dilution of the cultures before extraction is desirable if frothing difficulties are encountered. A large extractor is used with larger volumes of culture for extraction times of 24 to 72 hours.

After completion of the extraction, water is added to the ether extract, the ether is evaporated, and the aqueous sample is diluted to a known volume.

DETERMINATION

The apparatus used consists of a 300-ml. Kjeldahl flask fitted with a dropping funnel and a short glass condenser. A spray trap should be placed between the boiling flask and the condenser. Ground-glass connections are better than a rubber stopper, but if a stopper is used, it should be cleaned in boiling alkali before use. The tube at the delivery end of the condenser should be long enough to extend to the bottom of the receiver flask. The sample, containing 10 mg. or less of 2,3-butylene glycol, is pipetted into the Kjeldahl flask, 5 ml. of approximately normal sulfuric acid are added, and sufficient water to bring the total volume to about 50 ml. A pinch of talc is added to prevent bumping. In the 250-ml. Erlenmeyer receiver flask are placed 10 ml. of sodium bisulfite solution (12.5 grams per liter), made from fresh reagent and smelling strongly of sulfur dioxide. The end of the condenser should dip below the level of the bisulfite solution.

The burner is lighted, and as soon as the vapors reach the condenser 25 ml. of 0.01 *M* potassium periodate (2.3 grams per liter) are added by slow dropping from the dropping funnel. The rate of addition should be such that 25 ml. are added during 4 or 5 minutes. If periodate addition is begun before boiling begins, the first acetaldehyde produced will be mixed with air, and may not be completely absorbed by the bisulfite. If periodate addition is

delayed long after distillation is begun, butylene glycol will be lost in the distillate. Distillation is continued for 5 minutes after addition of the periodate has been completed. During the last minute of the distillation, the receiver flask is lowered, so that the end of the condenser no longer extends below the surface of the distillate. When distillation is stopped, the bisulfite adhering to the end of the condenser is rinsed into the flask.

Iodine solution (0.2 *N* is a convenient strength) is now added to the flask to oxidize the excess bisulfite. The iodine is run in carefully as the end point is approached. A drop or two of excess iodine is added, and, after the addition of starch indicator, the solution is adjusted to an accurate end point by weak thiosulfate. The sample is now ready for titration of the bound bisulfite, according to the procedure of Friedemann and Graeser (*2*). About 10 ml. of saturated sodium bicarbonate are added, and standard 0.01 *N* iodine from a buret is added rapidly, preferably at a rate such that the found bisulfite is oxidized as rapidly as it is liberated. When bisulfite liberation slows down, 5 ml. of bicarbonate are added, and the titration is carried to completion. One milliliter of 10% sodium carbonate is then added to ensure complete bisulfite liberation.

One equivalent of iodine corresponds to 0.25 mole of butylene glycol.

A blank determination should be run, and should give a titration of about 0.1 ml. of 0.01 *N* iodine. This blank value is applied as a correction to each determination.

RESULTS

As may be seen from Table I, recoveries by the oxidation and titration procedure average about 99%. The 2,3-butylene glycol was a sample of the meso compound, three times recrystallized. Its melting range (thermometer immersed in sample, rate of temperature rise, 0.2° C. per hour) was from 34.1° to 34.2°.

In Table II, the recoveries obtained in the extraction step are given. Each extractor (small vertical type) contained 5 ml. of 1% Difco yeast extract or 13% acid-hydrolyzed wheat mash. Each sample contained 110.2 mg. of glycol. The extraction time was 15 hours. Extraction from the clear medium was practically quantitative, but recovery from the hydrolyzed wheat mash, which contained a small amount of apparent glycol and much suspended material, was more erratic.

CORRECTION FOR ACETOIN

Most cultures containing 2,3-butylene glycol also contain acetoin, which will be extracted by ether. On periodate oxidation, it yields one molecule of acetaldehyde. When the acetoin content of the sample is determined by an independent method, a correction may be made for the interference caused. The acetoin method used in this laboratory is a modification of the method of Langlykke and Peterson (*3*). The foaming often encountered during the distillation in this method is obviated by dilution of the culture before distillation. To 10 ml. or less of distillate are added 2 ml. of *N* sodium hydroxide and 5 ml. of 0.02 *N* iodine. After 10 minutes, the sample is acidified and titrated with 0.005 *N* thiosulfate.

Table I. Recovery of Pure 2,3-Butylene Glycol

(Samples containing known amounts of pure butylene glycol were subjected to oxidation and titrated. They were not extracted with ether.)

Glycol in Sample	Glycol Recovered	Recovery
Mg.	*Mg.*	%
8.577	8.52	99.4
	8.47	98.8
4.233	4.180	98.8
	4.180	98.8
	4.203	99.3
	4.175	98.6
1.704	1.698	99.7
	1.698	99.7

Table II. Recovery of Butylene Glycol by Ether Extraction

Extractor No.	Glycol Extracted From yeast extract	Glycol Extracted From hydrolyzed wheat[a]
	%	%
1	99.8	96.0
	99.4	96.2
2	99.5	99.2
	99.5	99.2
3	99.8	96.5
	99.8	97.2
4	98.2	....
	97.0	....
Blank (no glycol added to medium)	0.0	1.0
	0.1	1.0

[a] Corrected for apparent glycol in hydrolyzate.

Table III. Determination of 2,3-Butylene Glycol by Direct Titration

Glycol in Sample	pH During Oxidation	Glycol Found	Recovery
Mg.		*Mg.*	%
4.208	[a]	4.172	99.3
		4.164	99.1
3.386	[a]	3.397	100.3
		3.396	100.3
1.693	[a]	1.712	101.1
		1.707	100.8
2.617[b]	[a]	2.594	99.2
		2.594	99.2
3.386	[a]	3.390	100.1
		3.388	100.1
3.386	2.6	3.400	100.4
3.386	4.8	3.402	100.4
3.386	7.0	3.900	115.0
	7.0	3.862	113.9
1.693	7.0	2.393	141.2
	7.0	2.443	144.2

[a] 0.067 *N* H_2SO_4.
[b] Sample consisted of acetoin, recovery calculated as acetoin.

DIRECT TITRATION OF 2,3-BUTYLENE GLYCOL

In the absence of other compounds oxidizable by periodate, 2,3-butylene glycol may be determined by titration of the excess periodate.

The sample, 10 ml. or less in volume, containing not more than 4 mg. of glycol, is pipetted into a 25 × 200 mm. test tube, 1 ml. of 1.0 *N* sulfuric acid and 5 ml. of potassium periodate solution (2.3 grams per liter) are added, and the contents of the tube are mixed. The tube is then heated for 10 minutes in a boiling water bath. After cooling, 5 ml. of 0.5 *M* of sodium dihydrogen phosphate solution are added. The contents of the tube are well mixed, and 1 ml. of potassium iodide solution (300 grams per liter) is added. The liberated iodine is titrated immediately with 0.005 *N* thiosulfate from a 25-ml. buret. The weak thiosulfate will keep for only a few hours, and is therefore made as needed by suitable dilution of stronger standard thiosulfate. The difference in titration between a tube containing the sample and a blank tube is a measure of the butylene glycol content of the sample. One milliliter of 0.005 *N* thiosulfate is equivalent to 0.2253 mg. of 2,3-butylene glycol. Acetoin interferes quantitatively, 1 mole of acetoin being equivalent to 1 mole of glycol.

In this procedure, the oxidation takes place in acid solution, but during the titration the pH is near 6.8. Under these conditions, periodate is reduced only to iodate, and large back-titrations are avoided. The titration of periodate in neutral solution has been applied to glycerol determination by Voris, Ellis, and Maynard (*4*).

From Table III, it may be seen that recoveries of slightly more than 100% are obtained with small samples, and that the presence of acid during the oxidation is essential. In the samples oxidized at definite pH values, the pH was held constant by suitable buffers. The titration step was always carried out at pH 6.8.

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Determination of Moisture in Whole Egg Powder

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A method of determining the moisture content of whole egg powder with a standard error in precision of 0.01% is presented. The equipment required is generally available, or can be easily made.

DURING a study of the drying of egg powder to low moisture levels (less than 1%), the need for a fast and reproducible moisture determination method soon became evident. The A.O.A.C. method (*2*) is relatively slow, requiring 5 to 6 hours' oven time, and it is impossible to reach a constant weight. The apparent moisture determined by this method increases at a rate of 0.06% per hour of oven time after 4 hours' initial heating.

It has been shown by Cleland and Fetzer (*3*) that correct preparation of the sample will allow moisture contents of thermal-sensitive products to be determined by the distillation method. In order to obtain satisfactory results by this method with dehydrated products at low moisture levels, the sample weight becomes excessive. The procedure of Makower and Myers (*4*) whereby the vapor pressure of the foodstuff is determined leads to a fair degree of reproducibility, but the results have to be converted by comparison with some direct method if they are to be recorded as per cent moisture. The determination of low moisture levels apparently requires the use of elevated sample temperatures, thereby introducing the factor of possible decomposition of the product. The Fischer titration method (*1*) has been reported (personal communication) to give excellent results on egg powder, but requires the services of an experienced analyst, since frequent standardization of reagents is necessary. A sensitive potentiometer with low current consumption is also required.

Previous experience in the drying of degraded starch products had indicated the value of a drying procedure utilizing a high-vacuum chamber with good heat transfer to the sample being dried. The apparatus shown in Figure 1 was devised and tests were run to determine the time and temperature required to obtain consistent results with a minimum of decomposition as indicated by a small continuous change in apparent moisture.

DESCRIPTION OF METHOD

The drying oven was made of two brass tubes of dimensions shown (Figure 1), all seams being silver-soldered and tested for leaks. It was considered advisable to use standard glass-stoppered weighing bottles, since these can be tightly closed to prevent moisture pickup while handling and weighing, are convenient to handle, and are light in weight. Outside ground cap weighing bottles 25 mm. in inside diameter and 50 mm. high were chosen, since a 3-gram sample of egg powder (apparent density 0.24 gram per cc., 15 pounds per cubic foot) could be easily placed in each bottle. These bottles had a cross-sectional area of 4.84 sq. cm. (0.75 square inch) and were high enough to prevent loss of sample when the pressure in the apparatus was suddenly reduced.

The holder illustrated in Figure 1, 10, maintained the bottles in a fixed position and was convenient to handle. It also allowed a large cross-sectional area to be maintained between the dry ice trap and the samples.

The top of the inner tube of the drying oven was connected to the dry ice trap using Pyrex tubing 17 mm. in outside diameter. Care was taken to maintain the full cross-sectional area of this tube at the bends. The connecting tubing was fastened to the U-tube by a short length of heavy walled rubber tubing, 1.25 cm. (0.5 inch) in inside diameter.

A standard 0.94-liter (1-quart) silvered Dewar flask held a mixture of methanol and dry ice. An excess of dry ice was added and the flask once filled needed no further attention for 8 hours or more. The U-tube trap was constructed of 17-mm. Pyrex tubing. It was found that this simple design of trap was stronger and just as effective as the more elaborate types. It was easy to clean. It did not plug up at the bottom, since incoming condensable vapors collected along the vertical arm above the bend, almost all condensation occurring in the incoming arm of the trap. The trap could be inspected while in operation, since the frozen moisture formed an easily observable layer in the tube, and the thickness of the collected layer could be estimated by looking down through the bend in the connecting tube. When, after several determinations, the layer of condensables reduced the internal diameter of the U-tube to about 0.6 cm. (0.25 inch), the trap was removed, the ice melted by holding under running water, and the trap dried with a small quantity of acetone. The dry ice–methanol mixture provided a convenient method of maintaining a low vapor pressure, since dry ice is now available from almost all ice cream manufacturers.

The system was connected to a Cenco Hyvac pump which was allowed to run continuously during a determination. A McLeod gage was used to check the pressure on the pump side of the dry ice trap. Without special precautions it was possible to maintain a pressure of 0.05 mm. of mercury consistently, and all determinations were made at this pressure.

The jacket of the drying oven contained a small quantity of water and was maintained at the boiling point by a small gas flame. The condenser at the top returned the condensate to the jacket. Water losses were found to be negligible. Other liquids could be substituted for water if desired to maintain higher or lower temperatures.

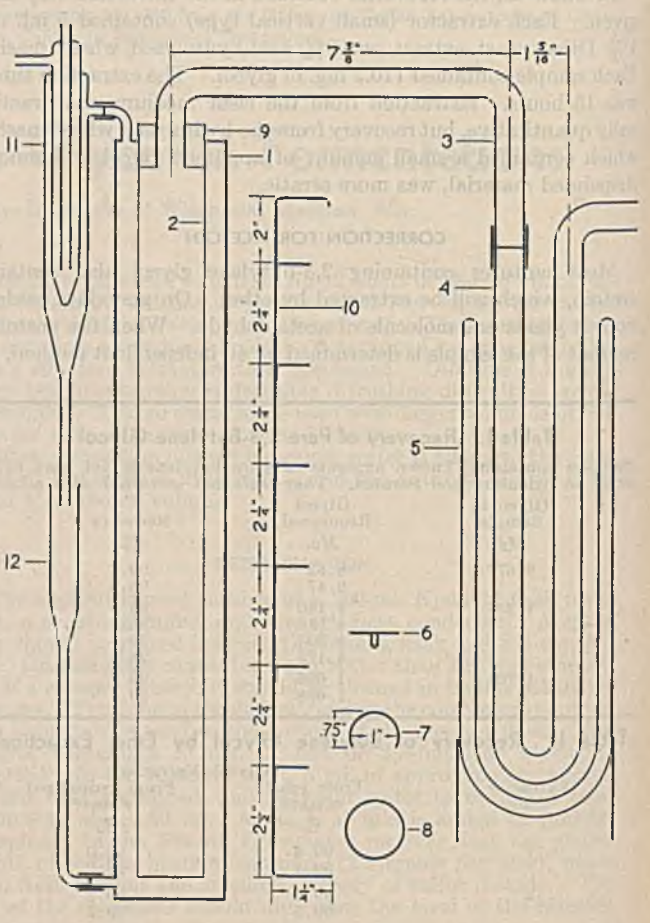

Figure 1. Apparatus for Determination of Moisture

(1) 17 × 2.5 inch O.D. 16-gage brass tube. (2) 16.5 × 1.5 inch O.D. 17/16 inch I.D. brass tube. (3) Connection tube, 17-mm. O.D. Pyrex tubing. (4) U-tube trap, 17-mm. O.D. Pyrex tubing, over-all length 12.375 inches. (5) 1-Quart wide-mouthed Dewar flask. (6) Side view of bottle holder rings, brass wire 18-gage. (7) Top view of bottle holder rings. (8) Top view of base of bottle rack. (9) No. 8 rubber stopper. (10) Bottle rack 14-gage brass wire, with rings soldered. (11) Condenser. (12) Level indicator and condensate return

EXPERIMENTAL PROCEDURE

Wash the weighing bottles, dry to constant weight (±0.0001 gram), and store in a desiccator containing anhydrous calcium sulfate. Place a 3.0 ±0.5-gram sample of powder in the bottle, quickly stopper, and weigh to 0.0001 gram. When working with egg powder of less than 1% moisture, the time of exposure of the egg powder to the atmosphere must be less than 10 seconds. A cork borer 15 mm. in diameter may be used to collect the sample. Push the borer to a depth of 30 to 40 mm. into the powder to be sampled, withdraw carefully, hold above the weighing bottle, and tap gently to cause the plug of powder to fall into the bottle. A minimum area of powder surface is exposed to the atmosphere by this procedure. The sampling time may be held to 10 seconds without undue difficulty.

Place from 1 to 6 samples in the drying oven rack after removing stoppers. Place stoppers in desiccator and place samples in the hot oven. Connect the dry ice trip, start pump, and note time when pressure reaches 0.05 mm. of mercury (about 2 minutes after starting pump). Maintain water at gentle boil as indicated by condensate dripping from condenser and maintain vacuum for 75 minutes. Release vacuum through the dry ice trap over a period of 30 seconds, so that moist air will not be sucked into drying oven. It is advisable to restrict the vacuum release with a length of fine capillary tubing to ensure a slow entry of air through the dry ice trap to the drying oven. Disconnect trap, remove samples, stopper immediately, and place in desiccator. Allow to stand 20 minutes, or until cold, and weigh to ±0.0001 gram. Empty weighing bottles, wipe thoroughly with clean absorbent gauze, reweigh, and store in desiccator.

SAMPLE PREPARATION

The results listed in Table I were obtained from three samples prepared as follows:

Samples of egg powder (300 grams) were sifted on a large sheet of paper, thoroughly mixed, resifted, mixed again, then placed in a 1-liter rubber-stoppered bottle. Six samples were run under each set of conditions. The time of treatment was taken from the time at which the pressure reached 0.05 mm. of mercury about 2 minutes after starting the pump.

Runs 1, 2, and 3 were made at 100° C., runs 5 and 6 at 78° C. The A.O.A.C. procedure was used for run 4. The results are collected in Table I and plotted in Figure 2.

DISCUSSION OF RESULTS

When weighings on 3-gram samples are made to 0.0001 gram, the precision of the moisture percentage is ±0.007 unit. The difference between two weighings, each made to ±0.0001 gram, is ±0.0002 gram, and for a 5% moisture sample loss in weight is 0.1500 gram.

$$(0.0002/0.1500) \times 100 = 0.13\% \text{ error}$$

$$5 \pm (5 \times 0.13\%) = (5 \pm 0.007)\%, \text{ for 1 sample}$$

The standard error of the arithmetic mean for six samples would be:

$$\sqrt{\frac{\Sigma (x^2)}{N(N-1)}} = \sqrt{\frac{0.000294}{6 \times 5}} = 0.0031$$

for 5 samples, 0.0035, and for 2 samples, 0.007, x is the deviation of any determination from the arithmetic mean. N is the number of determinations.

The standard errors of the experimental results for 60, 90, and 120 minutes fall between 0.021 and 0.001, the mean being 0.008. The experimental error is about twice the calculated weighing error, showing that errors in handling, heating, and cooling the weighing bottles are small and of no significance. When handling egg powder of low initial moisture, the chance of moisture pickup will be increased, and greater variations of the result will occur unless careful technique is used. It was found that a 0.6-cm. (0.25-inch) layer of egg powder changed from 1.06 to 1.49% moisture in a 5-minute period when exposed to a 50% relative humidity 21.11° C. (70° F.) atmosphere.

The rate of decomposition at 100° C. in this fast method in terms of apparent moisture increase is 0.055% per hour, estimated from the best straight lines through the 60-, 90-, and 120-

Table I. Determination of Moisture

Run No.	Heating Time, Minutes	No. of Samples	Mean Weight Loss, %	Standard Error
1	5	6	1.969	0.023
	10	6	2.392	0.034
	15	6	2.846	0.025
	20	6	3.081	0.027
	30	6	3.318	0.009
	60	6	3.383	0.010
	60	6	3.412	0.015
	90	5	3.467	0.007
	120	6	3.425	0.013
2	5	5	3.490	0.007
	10	5	4.814	0.075
	10	6	4.737	0.029
	15	6	5.155	0.019
	30	5	5.434	0.011
	60	6	5.528	0.005
	90	6	5.549	0.008
	120	6	5.593	0.008
3	60	6	5.175	0.008
	120	6	5.219	0.021
4	60	3	2.327	0.050
	120	3	2.929	0.050
	180	3	3.149	0.019
	240	3	3.333	0.020
	300	3	3.378	0.036
	390	3	3.453	0.021
5	60	6	5.382	0.008
	90	6	5.450	0.008
	120	6	5.451	0.006
6	60	6	5.041	0.001
	90	6	5.070	0.007
	120	6	5.088	0.005
	150	6	5.123	0.005

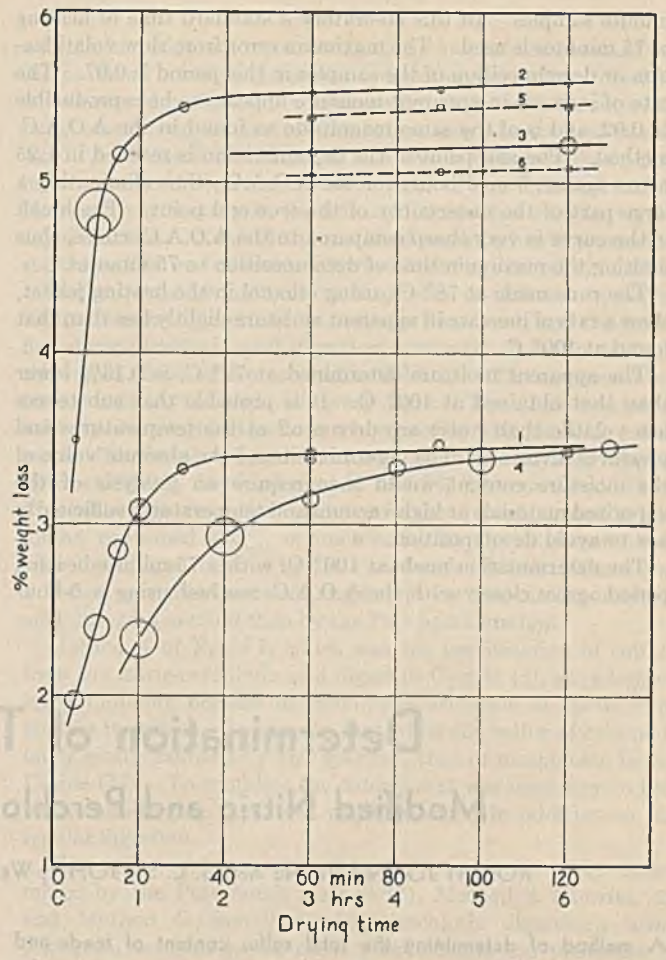

Figure 2. Moisture Determination

1 and 4. Fast method and A.O.A.C., respectively, run on same powder sample. 2 and 5. Fast method at 100° and 78° C., respectively, run on same powder sample. 3 and 6. Fast method at 100° and 78° C., respectively, run on same powder sample. Diameter of circles is four times standard error

minute samples. In this laboratory a standard time of heating of 75 minutes is used. The maximum error from slow volatilization or decomposition of the samples in this period is 0.07. The rate of increase in apparent moisture appears to be reproducible to 0.02, and is of the same magnitude as found in the A.O.A.C. method. The end point of the determination is reached in 1.25 hours against 5 or 6 hours for the A.O.A.C., thus eliminating a large part of the uncertainty of the true end point. The break in the curve is very sharp compared to the A.O.A.C. curve, thus limiting the maximum time of decomposition to 75 minutes.

The runs made at 78° C., using ethanol in the heating jacket, show a rate of increase in apparent moisture slightly less than that found at 100° C.

The apparent moisture determined at 78° C. is 0.13% lower than that obtained at 100° C. It is probable that substances less volatile than water are driven off at the temperatures and pressures involved. The determination of the absolute value of the moisture content would then require an analysis of the vaporized materials at high vacuum and temperatures sufficiently low to avoid decomposition.

The determination made at 100° C. with a 75-minute heating period agrees closely with the A.O.A.C. method using a 5-hour heating period; hence these conditions have been adopted as standard in this laboratory. The precision of this fast method gives a reproducibility of 0.01 percentage unit, and the variables such as temperature, vacuum, and moisture pickup can be easily controlled.

For routine moisture determinations using duplicate samples, weighings made to 0.001 gram will yield moisture percentage figures with a standard error of 0.07% moisture in the range of 0.5 to 5% moisture. However, for determinations on powder with moisture contents under 1%, greater precision of weighing is indicated.

ACKNOWLEDGMENT

The assistance of S. S. Jarrett in determining moisture contents is gratefully acknowledged.

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Determination of Total Sulfur in Feeds

Modified Nitric and Perchloric Acid Digestion Procedure

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A method of determining the total sulfur content of feeds and similar substances by destroying the organic matter and oxidizing the sulfur compounds to sulfates by digestion with nitric and perchloric acids is described. The sample is dissolved and partially oxidized by heating with concentrated nitric acid on a steam bath for 24 hours. The most important modification is the more complete oxidation of the sulfur compounds to sulfates, which is accomplished by gentle boiling with perchloric acid for about 15 hours. The results obtained agree with those by the Parr bomb method.

IN DETERMINING total sulfur in feeds, it is often desirable to use large samples of material containing small amounts of sulfur or to determine the sulfur content of the residue left on a filter paper. As the rapid Parr bomb method (*7*) cannot be used under such conditions, the use of nitric and perchloric acids for oxidizing organic matter appeared as a possible substitute for the Parr bomb sodium peroxide fusion method.

A combination of nitric and perchloric acids has been used to a considerable extent in this laboratory for the decomposition of biological materials preliminary to the quantitative determination of certain elements. Gerritz (*3*) used this method for digesting biological material for calcium and phosphorus determinations. Cook (*2*) digested feeds with nitric and perchloric acids to decompose the organic matter in preparation for the determination of manganese. St. John and Midgley (*8*) digested plant materials with nitric and perchloric acids as part of a rapid method for the determination of potassium in plant material.

In a preliminary study it was found that low results were obtained when sulfur was determined on a solution prepared by digesting the feed with nitric and perchloric acids as described by Gerritz (*3*) for the determination of calcium and phosphorus. Several modifications of this method were then investigated. Sulfur determinations were made by each method on samples of herring fish meal, soybean oil meal, and ground wheat.

METHODS

METHOD A. The nitric and perchloric acid digestion procedure developed by Gerritz (*3*) in this laboratory was used as a starting point, and all other methods were modifications of it. It consists essentially of adding 35 ml. of concentrated nitric acid to a 2.00-gram sample of feed in a 500-ml. Kjeldahl flask, and heating gently till the material is past the colloidal stage and goes into solution. Then 10 ml. of 70% perchloric acid are added and the solution is evaporated to perchloric acid fumes and then heated till it is colorless. This method was also found satisfactory in this laboratory for manganese (*2*) and potassium (*8*).

METHOD B. Evidence of Kahane and Kahane (*5*) indicates that low results may be caused by the loss of volatile sulfur compounds before they are oxidized. Sulfur may possibly be lost as hydrogen sulfide. One gram of copper nitrate was added to the samples before digestion in an attempt to fix the sulfur until oxidized to sulfate.

METHOD C. This is essentially the method of Wolesensky for the determination of sulfur in rubber (*9*). Essential differences from Method A are that the sample is digested on a steam bath with dilute nitric acid (1 to 1) solution until the reaction subsides before the addition of concentrated nitric acid. Five milli-

Table I. Total Sulfur in Feeds

(Comparison of some nitric-perchloric acid digestion procedures and Parr bomb method)

Method	Herring Fish Meal %	Soybean Oil Meal %	Ground Wheat %
A. Method first developed in this laboratory	0.634	0.286	0.109
B. Digestion in presence of $Cu(NO_3)_2$	0.475	0.326	0.099
C. Start digestion with 41% HNO_3, add HCl to drive off HNO_3 after digestion	0.545	0.302	0.114
D. Start digestion with HCl, HNO_3, and H_2O	0.692	0.336	0.123
E. Heat on water bath with HNO_3 for 24 hours	0.679	0.292	0.104
F. Boil perchloric acid gently for 15 hours	0.891	0.424	0.126
G. Combination of E and F	0.944	0.416	0.127
H. Parr bomb method	0.942	0.409	0.127

Table II. Total Sulfur in Feeds

(Comparison of Parr bomb and modified nitric-perchloric acid digestion methods)

Sample	Parr Bomb (H)	Nitric-Perchloric (G)	G/H × 100
	%	%	%
Feathers (0.5 gram samples, both methods)	2.093	2.034	97.2
Herring fish meal	0.958	0.950	99.2
	0.942	0.944	100.2
Pilchard fish meal	0.879	0.882	100.3
Crude casein	0.675	0.665	98.5
Powdered whey	0.309	0.325	105.2
Meat scraps	0.483	0.484	100.2
	0.434	0.432	99.5
Soybean oil meal	0.428	0.420	98.1
	0.409	0.416	101.7
	0.414	0.404	97.6
Cottonseed meal	0.403	0.418	103.7
Alfalfa	0.379	0.381	100.5
Oats	0.132	0.132	100.0
Wheat	0.127	0.127	100.0
Corn	0.128	0.125	97.7

Table III. Recovery of Sulfur of Cystine and Methionine

(By Parr bomb and nitric-perchloric acid digestion methods)

Method	Cystine A	Cystine B	Methionine
	%	%	%
Theoretical	26.69	26.69	21.50
Parr bomb	26.58	25.61	21.27
Nitric-perchloric (Gerritz, *3*)	25.65	24.46	1.55
Nitric-perchloric-modified (G)	25.76	25.24	20.65
$\frac{\text{Modified nitric-perchloric}}{\text{Parr bomb}} \times 100$	96.9	98.6	97.1

liters of concentrated hydrochloric acid are added after the perchloric acid solution has become colorless and it is again heated to perchloric acid fumes.

Method D. This is based on the micromethod of Jones (*4*), and consists of heating the material with 40 ml. of distilled water, 10 ml. of concentrated hydrochloric acid, and 30 ml. of concentrated nitric acid until in solution, and then proceeding as in Method A.

Method E. The sample is heated on the water bath with concentrated nitric acid for 24 hours, and perchloric acid is added after the material goes into solution. The nitric acid is then boiled off and the rest of the procedure of Method A followed.

Method F. The sample is treated with nitric and perchloric acids as in Method A. After the solution has been evaporated to perchloric acid fumes, it is boiled gently for about 15 hours. Additional perchloric acid is added when necessary to prevent evaporating to dryness.

Method G. This combines the modifications of Methods E and F and is essentially the method of Masters (*6*) with some modifications.

Method H. This is the Parr bomb method (*7*).

MODIFIED NITRIC AND PERCHLORIC ACID DIGESTION PROCEDURE FOR TOTAL SULFUR IN FEEDS

The data presented in Table I indicate that Method G was the only one of the nitric-perchloric acid digestion methods that gave results in good agreement with the Parr bomb method for the three samples studied. Therefore the following method was finally adopted for the determination of total sulfur in feeds by digestion with nitric and perchloric acids.

Weigh a 2.00-gram sample of the feed into a 500-ml. Kjeldahl flask, add 35 ml. of concentrated nitric acid, and heat on the steam bath till the feed goes into solution and the reaction subsides. Add 10 ml. of 70% perchloric acid and continue heating on the steam bath for a total of 24 hours. Heat the flask over a low flame till the nitric acid boils off and perchloric acid fumes are obtained. Adjust the burner so that the perchloric acid solution boils gently. Continue boiling for 15 to 16 hours, adding more perchloric acid when necessary to prevent evaporating to dryness. Usually about 5 or 10 ml. of additional perchloric acid are necessary. Cool, add 50 ml. of distilled water, and filter through a qualitative filter paper into a 600-ml. beaker. Wash the flask and filter paper well with distilled water. The volume at this stage is usually 250 to 300 ml. Add sodium hydroxide solution to the filtrate till neutral to methyl orange. Add 1 ml. of concentrated hydrochloric acid. Heat to boiling and add slowly 10 ml. of 10% barium chloride solution to precipitate the sulfate. Allow to stand for 24 to 48 hours, filter through a weighed Gooch crucible, and ignite at 800° C.

DISCUSSION

Some investigators have felt that the low results obtained by the previous nitric-perchloric acid digestion procedure (Method A) are caused by losses of volatile sulfur compounds through too rapid reaction or mechanical loss before they are oxidized to sulfate. A comparison of Methods A, F, and H (Table I) shows that incomplete oxidation accounts for most of the losses of sulfur. However, particularly in the fish meal, some loss was due to too rapid reaction. None of the methods employed to decrease the rapid reaction was of much value in increasing recovery of sulfur.

The total sulfur contents of a number of different types of feedstuffs were determined by both the Parr bomb and the modified nitric-perchloric acid digestion methods. A comparison of the values obtained is presented in Table II. The agreement between the two methods was very good. The nitric-perchloric acid digestion method gave an average of 99.98% as much sulfur as the Parr bomb method for the 16 samples analyzed, ranging between 97.2% for feathers and 105.2% for powdered whey. Materials high in sulfur, such as feathers, cystine, and methionine, contained 97.5% as much sulfur by the nitric-perchloric acid digestion method as by the Parr bomb method. There was a better agreement between duplicates by the nitric-perchloric acid digestion method than by the Parr bomb method.

Method A of Table I, which was the precipitation of sulfate from the nitric-perchloric acid digest of Gerritz (*3*), gave low results, probably because of incomplete oxidation of sulfur compounds to sulfate. It appears that while the sulfur of cystine is fairly easily oxidized by this method, that of methionine is not (Table III). To complete the oxidation it was necessary to boil for a considerable time with perchloric acid in addition to the regular digestion.

The sulfur contents of cystine and methionine were determined by the Parr bomb method (*7*), Method A (Gerritz, *3*), and Method G (modified nitric-perchloric digestion), using 0.05-gram samples in all cases. The results are presented in Table III. Although the Parr bomb method did not give a sulfur content as high as the theoretical in any of the amino acids, it probably represents the true sulfur content. Cystine A and the methionine were commercial preparations with no statement of purity. Cystine B was prepared from hair in the laboratory and was not highly purified. The modified nitric-perchloric acid method gave 96.9 to 98.6% as much sulfur on these compounds as the Parr bomb method. The previous nitric-perchloric acid method of digestion gave a good recovery from cystine but very little from methionine. This agrees with the findings of Callan and Toennies (*1*) for the alkaline permanganate method.

The total time required for making a determination by the nitric-perchloric acid digestion method on a set of six samples in duplicate was 6 days from the time the samples were weighed out till the results were obtained. This contrasts with 4 days by the Parr bomb method. However, the nitric-perchloric acid digestion method required about 5 hours less of the analyst's actual working time than the Parr bomb method.

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Determination of Vitamin A in Dehydrated Eggs

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A spectrochemical method for the determination of vitamin A in dehydrated eggs is described. The procedure involves careful control of analytical conditions, followed by the use of a correction factor required because of the absorption of ultraviolet radiation by the carotenoid pigments present. The correction must include the effects of isomerization of the pigments caused by the analytical process, and has been determined on the basis of the two principal pigments present, luteol and zeaxanthol. The absorption spectra of these two pigments, in absolute ether, and the specific absorption coefficients at their wave lengths of maximum and minimum absorption are presented.

THE increased emphasis on the nutritional value of foods and food products has increased, among other things, the need for adequate methods of analysis for vitamin A. Biological methods are both time-consuming and expensive. Chemical methods seem to be limited at present to two procedures: the well-known Carr-Price (*2*) antimony trichloride reaction, or analysis based on the absorption of ultraviolet radiation by the vitamin A. In the case of dehydrated eggs, a product that is being studied in this laboratory, the pigments present influence the results, and adequate correction factors are required. This paper presents a method of analysis based on the ultraviolet absorption of vitamin A, with a suitable correction for the presence of the two principal carotenol pigments. Duplicate samples agree within 10%, and a few samples on which bioassays were available give reasonable checks. Extraction procedures are similar to those used for the extraction of vitamin A from other products.

PROCEDURE

EXTRACTION OF VITAMIN A. A 10-gram sample of dehydrated egg is placed in a Waring Blendor and extracted for 10 minutes with 100 ml. of peroxide-free ether. The sample is filtered on a Büchner funnel and washed with several small portions of ether. The egg powder is then replaced in the Blendor and re-extracted with 60 ml. more of ether for 2 minutes. A third extraction for another 2 minutes is usually required. This extraction procedure removes all the vitamin A and practically all the yellow pigments.

PREPARATION OF EXTRACT FOR ANALYSIS. The combined ether extracts are placed in a 500-ml. round-bottomed flask, to which a few glass beads have been added to prevent bumping, and evaporated on a steam cone, under the reduced pressure produced by a water pump, to a final volume of 15 ml. (It is important that this evaporation be carried to the same volume each time.) Twenty milliliters of 95% methanol and 5 ml. of a saturated aqueous solution of potassium hydroxide are added to the residue in the flask, and the mixture is heated under reflux for 10 minutes. The sample is cooled immediately; 40 ml. of water are added and the contents are then transferred to a 500-ml. separatory funnel. The flask is rinsed with an additional 40 ml. of water, followed by rinsings with two 25-ml. portions of ether, which are also added to the contents of the funnel. The methanol-water solution of vitamin A is then extracted with 25-ml. portions of ether until the ether layer is colorless. It has been shown that the absence of yellow color in the ether phase is an indication of complete extraction of vitamin A.

The ether extract is washed with water until the wash is neutral to litmus, and then dried over anhydrous sodium sulfate. The extract is filtered through sodium sulfate into a volumetric flask. A crystal-clear, yellow filtrate should result.

SPECTROPHOTOMETRIC ESTIMATION OF VITAMIN A. The sample is made up to volume (250 ml.), and its optical density is determined on a Beckman (*3*) spectrophotometer at 326 mμ for vitamin A and at 450 mμ for total yellow color. Fifteen per cent of the density at 450 mμ is subtracted from the density at 326 mμ as a correction for absorption at 326 mμ due to the yellow pigments present. The vitamin A present may be calculated by means of the following equation:

$$\text{Micrograms of vitamin A per gram} = \frac{D_{326} - 15\%\ D_{450}}{0.176} \times \frac{\text{volume in ml.}}{\text{weight in grams}}$$

DISCUSSION

The extraction of the ether-soluble fraction from dehydrated eggs was somewhat more difficult than from many other food products. Several methods of extraction were tried before the method described was finally chosen. The Waring Blendor treatment apparently removed all the vitamin A. Further extraction produced no increase in the quantity of vitamin A removed.

Evaporation of the ether extract must be carefully controlled and brought to the same final volume in each case, since carotenoid-type pigments isomerize under the influence of heat (*9*, *15*). The isomerization of the pigments produces changes in their spectral absorption curves, which affect the absorption in the ultraviolet region where correction for their presence is necessary. The time of saponification is held constant for the same reason.

Studies on the pigments of eggs (*6*, *7*, *8*) show that, although pigmentation may be controlled by feeding, the pigments in eggs produced by hens on a normal diet contain over 90% luteol and zeaxanthol. The remaining pigments are primarily cryptoxanthol and carotene.

Several different samples of dehydrated eggs were extracted with ether and the pigments adsorbed on a column of 1 part, magnesia (Micron brand No. 2641) and 2 parts Hyflo Super-Cel. The column was developed with a mixture of 12% acetone in Skellysolve B. Five bands were produced, four of which were identified as the pigments named above; the fifth, which was held tightly at the top of the column, was undoubtedly a mixture of very small amounts of oxidized material. The four identified pigments were quantitatively determined by removing the column, cutting out the various bands, and eluting the pigment with acetone. Skellysolve B was then added and the acetone removed by washing with water. The extract was dried over anhydrous sodium sulfate and made up to a convenient volume. The amounts of pigments present were then determined, using the specific absorption coefficients and wave lengths of maximum absorption as given by Zscheile *et al.* (*18*). Slight errors may be expected in the cases of luteol and zeaxanthol, since the data of Zscheile were obtained in an ethanol solution. No coefficients were available for these two pigments in petroleum ether or hexane. A comparison of the total pigment present as compared to the sum of the fractions eluted from the column indicated some loss (10 to 15%), which is assumed to be distributed proportionally through the components.

In all cases the sum of the concentrations of luteol and zeaxanthol accounted for more than 90% of the total pigments, luteol being present in the larger amounts. The amount of luteol in different samples varied from 63 to 76% of the total pigment, while the zeaxanthol varied from 32 to 20%. The cryptoxanthol amounted to 3 to 5% and the carotene to 2 to 4% of the total. The remainder was in the band at the top of the column.

Since the luteol and zeaxanthol accounted for approximately 90% of the total pigment, corrections for their presence were obtained. The other pigments were not included in the establishment of the correction factor because of the small amounts present. It has been shown, however, that β-carotene affects analytical data in the same general way (*10*). In order to obtain corrections, luteol was crystallized from alfalfa meal and zeaxanthol from dehydrated eggs, the crystallizations and separations being

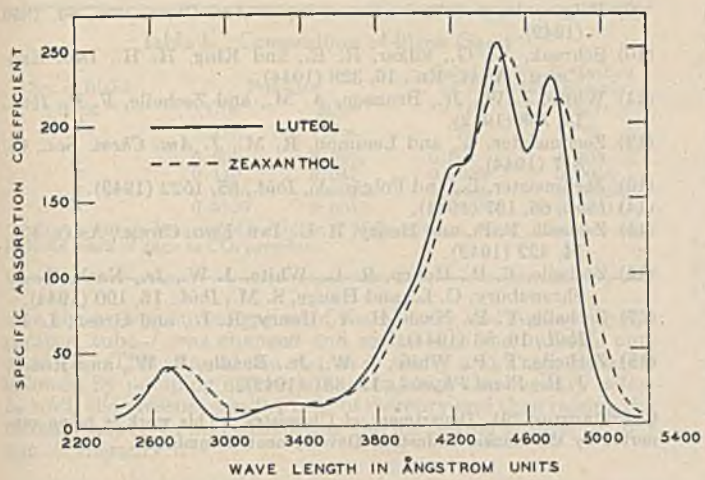

Figure 1. Absorption Spectra of Luteol and Zeaxanthol in Absolute Ether

Table I. Absorption Values of Luteol and Zeaxanthol in Ether

	Luteol		Zeaxanthol	
	λ (mμ)	α	λ (mμ)	α
Maxima	473.5	230	476.0	217
	444.5	254	450.0	246
	422.0	173	...	...
Minima	460.0	181	468.0	199
	425.0	172	...	...

Table II. Effect of Analytical Procedure on Ultraviolet Absorption of Luteol and Zeaxanthol

Pigment	Treatment	D_{451} mμ	D_{326} mμ	$\frac{D_{326}\ m\mu}{D_{451}\ m\mu} \times 100$
Luteol	None	0.675	0.042	6.2
	Through analytical procedure	0.778	0.122	15.7
		1.500	0.191	12.7
		0.948	0.150	15.8
Zeaxanthol	None	0.715	0.045	6.3
	Through analytical procedure	0.165	0.028	16.9
		0.118	0.020	16.9
½ luteol plus ½ zeaxanthol	Through analytical procedure	0.770	0.116	15.1
		0.635	0.095	15.0

carried out as described by Zscheile *et al.* (*18*). After recrystallization their absorption spectra were determined, in ether, from 510 to 240 mμ. These absorption curves are similar to those of White, Brunson, and Zscheile (*11*) over the range used, although they should not be compared too closely because different solvents were used. The wave lengths of the maxima and minima together with the corresponding specific absorption coefficients, in diethyl ether, are presented in Table I.

It will be seen in Figure 1 that the curves intersect near 450 mμ and are close together at 326 mμ, the wave length at which the vitamin A maximum occurs. The absorption at 326 mμ is about 6.2% of the absorption at 450 mμ. This is about the same as the ratio reported by Deuel *et al.* (*4*), which they obtained on a sample of luteol furnished by Zechmeister and used to establish a correction for egg pigments. They used a mixed solvent of low-boiling petroleum ether and isopropanol, while the authors' data were obtained with ether as the solvent.

However, 6.2% should not be used as the correction for these pigments, since isomerization raises the absorption peak in the ultraviolet. Zechmeister (*12, 14*) has recently presented data on luteol and zeaxanthol showing this to be true of these two pigments as well as others. To determine the extent of this effect, a sample of pure luteol was carried through the analytical procedure and the ratio of the absorption readings at 450 and 326 mμ determined. The same procedure was followed with zeaxanthol and a mixture of the two pigments in the approximate proportions in which they occur in eggs. The results, tabulated in Table II, show that the true correction is about 15% of the absorption at 450 mμ. This correction is valid only if the heating times and procedures used are those given here. The 15% correction obtained for these pigments is very similar to a correction applied to butterfat by Baumann *et al.* (*1*) several years ago. Longer periods of heating cause additional isomerization and consequently would affect the reading.

The absorption coefficient of 0.176 in the equation used for the calculation of the vitamin A concentration has been determined on a sample of crystalline vitamin A generously furnished by Distillation Products, Inc. This figure is close to the average obtained by Zscheile and Henry (*15*), although Zscheile *et al.* (*17*) later report another sample as having a value of 0.1825, with an absorption peak at 324 mμ. The absorption maximum obtained with this sample of vitamin A occurred at 326 mμ when the vitamin A was dissolved in ether.

The recovery of vitamin A was fair. Recoveries of added vitamin A averaged slightly over 90%. An ether solution of vitamin A alcohol carried through the procedure alone gave slightly higher recovery percentages.

The usual precautions regarding light were taken, although amber glassware, as recommended by Embree (*5*) was not available. All steps in the procedure were carried out in a darkened room. Sunlight was excluded entirely and diffuse light used for illumination. Whenever possible, the samples were placed in a cupboard, in the dark. Spectrophotometric readings were taken the day of extraction, or kept in refrigeration until read. The usual checks on solvents also were made. Ether was free of peroxides and the alcohol free of aldehydes. Glassware was thoroughly cleaned between analyses. Special care is needed to remove all fatty materials which might become rancid and destroy vitamin A.

A bioassay comparison with the proposed spectrophotometric procedure on the three samples which were available showed fair agreement (see Table III). Similar agreement was obtained by Zscheile *et al.* (*16*) on samples of butterfat analyzed by spectrophotometric methods, using a somewhat similar arbitrary correction factor. The comparison with the bioassay is made assuming that the vitamin A has a potency of 4,300,000 U.S.P. units of vitamin A per gram. No determination of the additional vitamin A effects of other substances present is included.

Table IV presents typical data obtained on several samples analyzed by the proposed method. Although the correction required because of the presence of the yellow pigments is large, the

Table III. Comparison of Bioassay and Spectrophotometric Analysis

Sample	Bioassay I.U./g.	Spectrophotometric Analysis I.U./g.
1	36.3	35.1
		33.1
2	31.6	35.4
		35.5
3	58.5	49.5
		46.8

I.U./g. = 4.3 × γ/g.

Table IV. Typical Spectrophotometric Data Obtained by Described Procedure

Sample	D_{450} mμ	D_{326} mμ	Correction for Carotenols (15% D_{450} mμ)	Corrected D_{326} mμ	Micrograms of Vitamin A per Gram
1	0.640	0.183	0.096	0.087	12.3
	0.614	0.184	0.093	0.091	12.9
2	0.560	0.157	0.084	0.073	10.4
	0.553	0.158	0.082	0.076	10.8
3	0.421	0.121	0.063	0.058	8.2
	0.405	0.116	0.061	0.055	7.8
4	0.155	0.081	0.023	0.058	8.2
	0.160	0.092	0.024	0.068	9.6
5	0.143	0.097	0.021	0.076	10.8
	0.145	0.089	0.021	0.068	9.6

method gives reasonable, reproducible results. In a series of 120 samples under various treatments and storage conditions, values ranging from 13.6 to 6.3 micrograms per gram were obtained, well within the range of values obtained by other methods of analysis.

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Determination of Carbon

Simplification of Low-Pressure Combustion Apparatus

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The interference of water vapor in the apparatus for the low-pressure combustion method of determining carbon in iron and steel has been investigated and changes in operating technique have minimized this interference. The equipment has been simplified further, resulting in a greater speed of manipulation. In over 600 determinations made with the new equipment the results have shown good agreement with those obtained on earlier forms of apparatus.

THE composition of the blank gases obtained in the low-pressure combustion equipment described by Murray and Ashley (*2*) has been determined by means of vapor pressure curves. A considerable fraction of this gas was found to be water vapor which moved from the dry ice trap to the liquid nitrogen trap during the pumping period.

The authors have replaced the complex mercury cut-off system in the previous apparatus (*2*) with a single stopcock. Two carbon dioxide measuring systems are used with one combustion vessel. These changes have decreased the time required for a determination, so that 10 to 15 samples can be run in an 8-hour shift on one unit of this type.

ANALYSIS OF BLANK GASES

A carbon analysis unit as described by Murray and Ashley (*2*) was equipped with a single trap at T_5. Vapor pressure curve analysis of gases frozen out in this trap was made by a procedure similar to that described by Sebastian and Howard (*3*).

The vaporization curve of mixtures of carbon dioxide, sulfur dioxide, and water was measured in the equipment in order to prove that these gases could be identified. This mixture was introduced into the system by warming sodium bicarbonate and sodium bisulfite which were placed in a branch of a loading arm prior to evacuation of the system. Curves obtained for two different mixtures of these gases are shown in Figure 1.

A new platinum crucible with beryllia lining crucible was used for the investigation of the origin and composition of the blank. A vapor pressure curve analysis was carried out on each blank on this crucible assembly. These blanks, run according to the operating procedure of Murray and Ashley (*2*), were found to contain carbon dioxide and water vapor, as shown in Table I. No evidence of the presence of other gases was found. From these and similar data it appears that water vapor is responsible for from $^1/_3$ to $^1/_2$ of the blank when it is in the low range used for an actual carbon analysis (blank <0.001% carbon on a 0.5-gram sample).

During collection of these blank gases, the combustion vessel and dry ice trap, T_4, were connected during the entire pumping period until the pressure had been reduced to 10^{-5} mm. of mercury. Gurry and Trigg (*1*) avoid the transfer of water vapor from the dry ice trap to the liquid nitrogen trap by closing off the former when the pressure has been reduced to 0.1 mm. of mercury and then exhausting the actual measuring system to 10^{-5} mm.

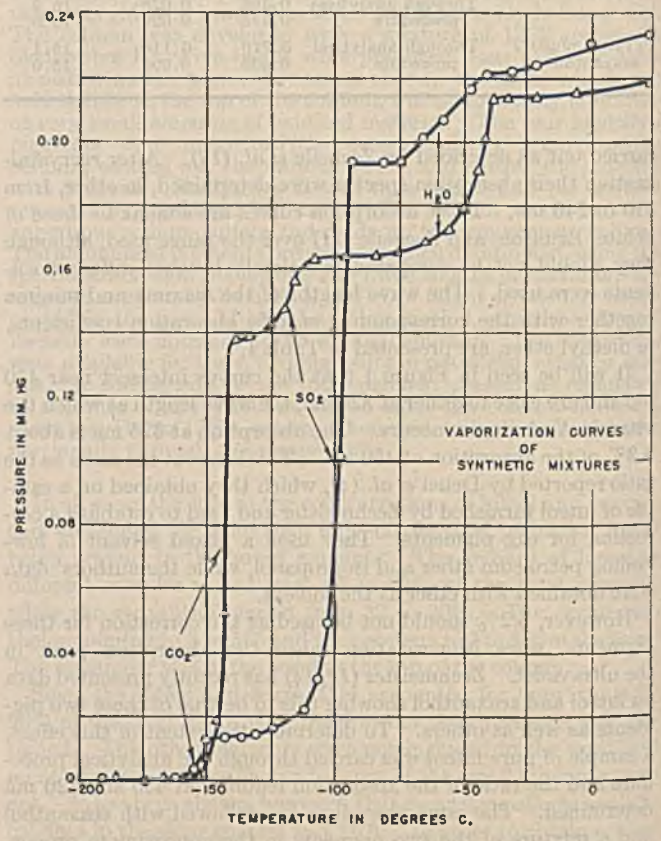

Figure 1

Table I. Composition of Blank Gases

No. of Blank Run	Pressure CO_2	Pressure H_2O[a]	Calcd. as Wt. % Carbon on 0.5 Gram Sample CO_2	Calcd. as Wt. % Carbon on 0.5 Gram Sample H_2O[a]
	Mm.	*Mm.*	%	%
1	0.598	0.097	0.021	0.003
2	0.110	0.047	0.0039	0.0016
3	0.0735	0.025	0.0026	0.00088
4	0.0100	0.0048	0.00035	0.00017

[a] Not accurate because of behavior of H_2O vapor in gage. Pressure read on same mark of gage as CO_2 pressure.

In the present investigation of the Murray and Ashley (*2*) apparatus, tube *J* was changed and sealed into the multiple cut-off at L_2. This allowed the system of Gurry and Trigg (*1*) to be followed by pumping after combustion, with the mercury below L_3, until the pressure was 0.1 mm. of mercury and then raising the mercury above L_2 and exhausting the measuring system to 10^{-5} mm. of mercury.

In Figure 2 the effect of the water vapor transfer from trap T_4 to trap T_5 is illustrated by vapor pressure curves.

Curve *A* was obtained from a blank in which the combustion vessel and T_4 were pumped to 10^{-5} mm. of mercury (20 minutes) along with the measuring system. This curve shows that water vapor does transfer from T_4 to T_5 under these conditions of pumping.

Curve *B* represents another blank in which pumping of T_4 was stopped when the pressure reached 0.1 mm. of mercury and only the measuring system was evacuated to 10^{-5} mm. This curve shows that this method of operation eliminates the transfer of any appreciable quantity of water vapor into T_5 from T_4.

The series of *C* curves was obtained, after exhausting the gas of *B*, by pumping the entire system for 30 minutes with T_4 and T_5 cooled to their indicated temperatures. Thus these curves represent the transfer of water vapor from T_4 to T_5 during a 30-minute pumping period. C_1 was read on the high-compression mark of the McLeod gage, C_4 on the low-compression mark, and C_2, C_3 on intermediate marks.

DESIGN OF NEW APPARATUS

Since the apparatus described by Murray and Ashley (*2*) contained two stopcocks per unit, it seemed worth while to attempt the introduction of one more stopcock and thus eliminate the complex mercury cut-off system entirely. Shepherd (*4*) pointed out that no difficulty had been encountered from stopcocks in the previous apparatus (*2*) and trap T_4 should remove any contaminating material (derived from the grease) from the oxygen entering the combustion vessel.

The new apparatus is shown diagrammatically in Figure 3. The oxygen purification system is similar to that used previously (*2*). Only the *A* measuring system is shown in the diagram, but the *B* system is identical in construction. Stopcocks S_{3A} and S_{3B} replace the mercury cut-offs in two units of the earlier apparatus. Bulb X_A is of such a volume that the total calibrated volume ($S_{3A} - S_{4A}$) is about 500 ml. thus making it possible to handle samples containing 0.001 to 0.10% carbon in this single intermediate volume rather than the two volumes used previously. The two measuring systems, *A* and *B*, and one combustion vessel constitute a unit. In the authors' laboratory two units of this type are built on one 2 × 6 foot table with the oxygen purification system serving both units, as indicated by the dotted tube above S_2.

All stopcocks are hollow-plug precision-ground obtained from Eck and Krebs, New York, N. Y. S_1, S_2, S_3, and S_4 are 10- to 12-mm. bore. This large size is used because there is less danger of leaking and because the large bore does not plug easily with grease and stop the pumping of gases at low pressures. S_5 and S_6 are of smaller bore (4 to 6 mm.). All stopcocks are greased with Apiezon L.

Calibration of the known volume is made in the manner described by Murray and Ashley (*2*).

OPERATION OF EQUIPMENT

(Reference is made to measuring system *B*, although it is not shown in Figure 3.)

With S_1, S_{5A}, and S_{5B} closed, evacuate the entire system to 10^{-5} mm. of mercury pressure. Cool T_1, T_2, T_{5A}, and T_{5B} with liquid nitrogen and T_3 with dry ice–acetone mixture. Close

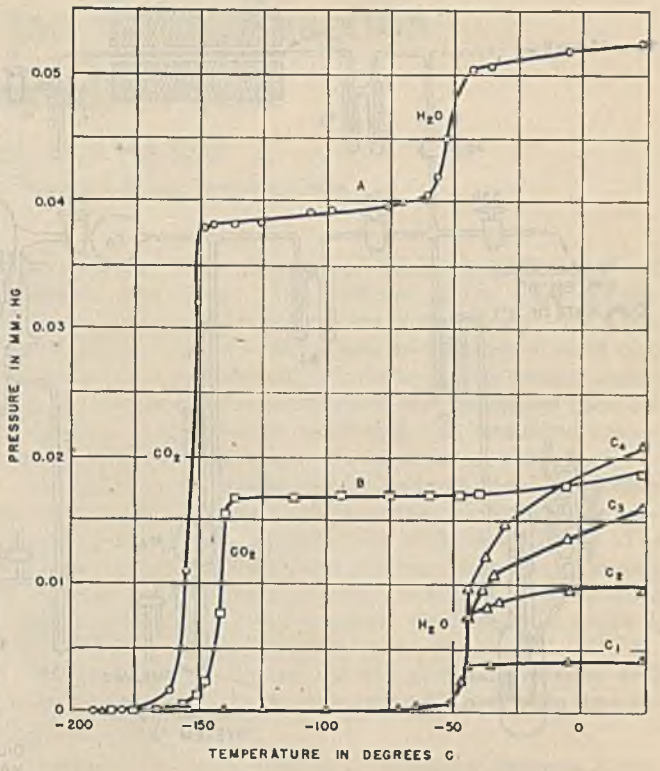

Figure 2

S_{3B} and S_{4A}, then admit oxygen slowly through S_1 until the pressure in the combustion vessel and measuring system *A* is 15 to 20 cm., as indicated by depression of the mercury column below gage M_A. Close S_2 and cool T_{4A} with liquid nitrogen. Burn the sample in the manner described previously (*2*), then open S_{4A} very slightly and pump the gas slowly from the combustion vessel through T_{4A}. The rate of removal of gas will be indicated by the rate of mercury rising from the reservoir below M_A, and must be slow, so that all the carbon dioxide is frozen out in T_{4A}. When the mercury ceases to rise below M_A, open S_{4A} completely and pump until the pressure is 0.1 mm. (The time required for this initial pumping down to 0.1-mm. pressure is usually about 5 minutes.) Close S_{3A} and evacuate measuring system *A* to 10^{-5} mm. of mercury pressure (time about 5 minutes). Close S_{4A} and expand the carbon dioxide into the known volume $S_{3A} - S_{4A}$. Measure the pressure of this gas at room temperature, then open S_{4A} and exhaust measuring system *A*.

As soon as S_{3A} is closed after burning the first sample, open S_{3B} and close S_{4B}. Admit oxygen to the combustion vessel and measuring system *B* and burn another sample while system *A* is being pumped and the gas measured. By the time system *A*

Table II. 8-Hour Record of Analyses Showing Blanks

(New crucible used, all blanks recorded)

No. of Burning	Measuring System Used	Sample	% Carbon
1	A	Blank	0.0246
2	B	Blank	0.0006
3	A	Blank	0.0004
4	B	Blank	0.0003
5	A	Fe-Ni-Co alloy[a]	0.0205
6	B	Fe-Ni-Co alloy	0.0093
7	A	Fe-Ni-Co alloy	0.0079
8	B	Fe-Ni-Co alloy	0.0274
9	A	Blank	0.0003
10	B	Si steel[b]	0.0044
11	A	Blank	0.0003
12	B	Si steel	0.0041
13	A	Si steel	0.0042
14	B	Si steel	0.0056
15	A	Blank	0.0004
16	B	Blank	0.0005

[a] Four samples of Fe-Ni-Co were different.
[b] Four samples of Si steel were same material.

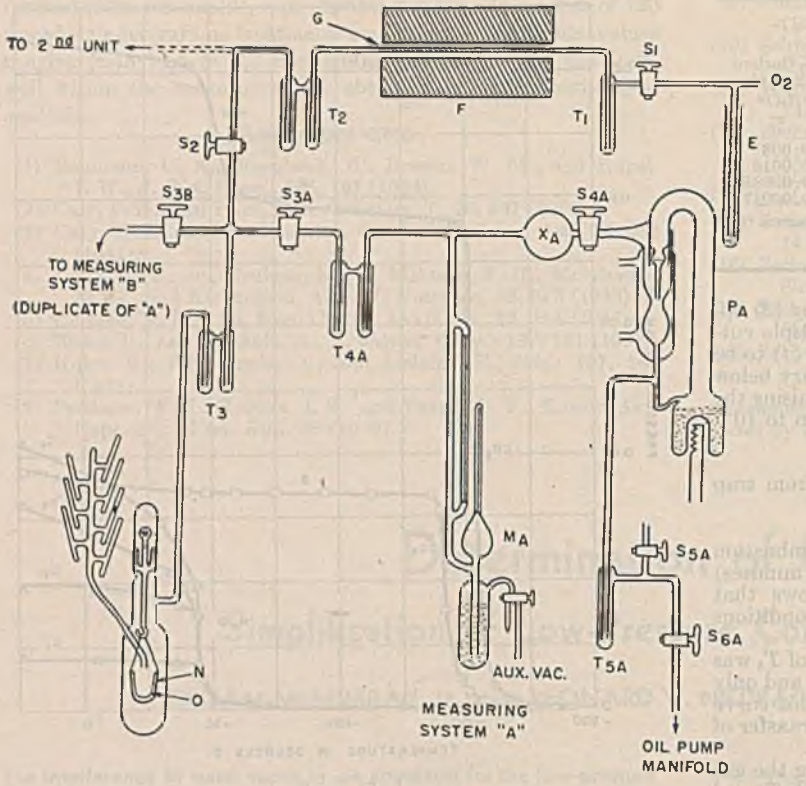

Figure 3. Apparatus

is ready for the third sample, system B will be pumping with S_{1B} closed, so that the combustion vessel will be ready for A.

Using this scheme of operation, about 0.1% of the gas from the first sample remains in the combustion vessel when oxygen is added for burning the second sample. If the first sample contained 0.1% carbon, the carbon dioxide remaining in the combustion vessel would correspond to 0.0001% carbon, which is negligible as a loss from the first sample or as an addition to the second sample. Even after running 10 samples in this manner the accumulation of carbon dioxide in the combustion vessel would be equivalent to only 0.0001001% carbon. Since the samples usually contain less than 0.02% carbon, this error is entirely negligible.

Interference by water transferring from T_3 to T_{4A} or T_{4B} has not been encountered since this method of pumping has been employed.

ROUTINE OF OPERATION

One unit with two measuring systems can be handled by one operator very easily. It is possible to determine carbon on 10 to 15 samples in 5 to 6 hours in this manner.

The routine established has been that of preparing a loading arm containing 10 to 15 samples and sealing this onto the combustion vessel late in the afternoon. The apparatus is pumped out and trap T_3 flamed with a torch for a few minutes while pumping. The next morning blanks are run (2 or 3 as required) and then all the samples analyzed. By mid-afternoon the loading arm is empty and the apparatus is shut down and air admitted. Another loading arm filled with samples is sealed on, the crucible changed if necessary, and the apparatus exhausted and T_3 flamed. By following this routine it has been possible to turn out 10 to 15 analyses every day without difficulty.

Flaming of trap T_3 after each time that air is admitted to the apparatus (or once daily) is important to prevent water vapor and sulfur compounds from accumulating in this trap.

BLANK

The blank on this new equipment is easily reduced to a value equivalent to 0.0005% carbon or less on a 0.5-gram sample. The data given in Table II are representative of normal operation of a unit, although more blanks and fewer samples were run in this case than usual in order to illustrate the maintenance of a low blank. These data were accumulated in a regular 8-hour shift.

RESULTS WITH REFERENCE SAMPLES

The data given in Table III represent all the results obtained on two reference samples during the first six weeks of operation of the new apparatus. One of the reference samples is Bureau of Standards Sample 55a, a very pure open-hearth iron, and the other is a 3% silicon steel sample which has been used by the authors for reference.

The average value of 0.0108% carbon found on B. of S. 55a is to be compared with the value 0.0121% carbon previously reported from this laboratory (*2*) and the values 0.0108% carbon reported by Wooten and Guldner (*5*) and 0.0116 reported by the U. S. Steel Corporation (J. B. Austin) (*2*). It is evident that this lower value obtained by the new method is in better agreement with the values obtained by other users of the low-pressure combustion method than that obtained at Pittsfield on the previous apparatus of Murray and Ashley (*2*).

The one very high value (0.0086% carbon) found on the silicon steel sample probably resulted from contamination and was rejected in calculating the average and the average deviation.

These data of Table III indicate that the new simplified apparatus yields satisfactory results. The values reported have not been corrected for the blank because it is difficult to establish a fixed blank to be used for such a correction. A blank less than 0.001% carbon and a precision of ±0.001% carbon has been considered satisfactory for the routine analysis of this laboratory, and the new apparatus meets these requirements very satisfactorily.

Table III. Carbon in Reference Samples

(Not corrected for blank)

B. of S. Sample 55a %	Silicon Steel %	B. of S. Sample 55a %	Silicon Steel %
0.0106	0.0035	0.0108	0.0032
0.0106	0.0037	0.0103	0.0045
0.0116	0.0038	0.0113	0.0044
0.0104	0.0034	0.0113	0.0042
0.0104	0.0034	0.0107	0.0044
0.0110	0.0042	0.0121	0.0041
0.0111	0.0045	0.0109	0.0056
0.0107	0.0032	0.0106	0.0043
0.0105	0.0049	0.0103	0.0043
0.0103	0.0039	0.0098	0.0036
0.0103	0.0051	0.0100	0.0044
0.0111	0.0032	0.0103	0.0028
0.0109	0.0039		0.0052
0.0114	0.0033		0.0038
0.0104	0.0031		0.0036
0.0119	0.0086[a]		0.0036
0.0104	0.0037		0.0029
0.0107	0.0032	Av. 0.0108	Av. 0.0039
		Av. deviation 0.00043	Av. deviation 0.00055

[a] Value rejected, not used in calculating average or average deviation.

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Qualitative Study of the Color Reaction of Phosphomolybdic Acid

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THIS short paper reports the color reaction of phosphomolybdic acid with six sugars, twelve metals, and thirteen other reducing agents.

REAGENT. Phosphomolybdic acid solution (test solution), 1 gram of $P_2O_5.24\ MoO_3.xH_2O$ (Schering-Kahlbaum), dissolved in 100 ml. of water.

Table I. Color Reaction of Phosphomolybdic Acid and Molybdate with Sugars

Sugar	Test Solution (3 Ml.)	Test Solution (3 Ml.) + 3 *N* H_2SO_4 (1 Ml.)[a]	1% Ammonium Molybdate (3 Ml.)[b]	1% Ammonium Molybdate (3 Ml.) + 3 *N* H_2SO_4 (1 Ml.)[b]
Glucose	Light green	Light green	None	Blue
Galactose	Light green	Green	None	Blue
Fructose	Bluish green	Deep bluish green	None	Deep blue
Maltose	Green	Green	Blue	Bluish green
Lactose	None	Light green	None	Blue
Sucrose[c]	Green	Deep bluish green	None	Deep blue

[a] Reagent retained yellow color after boiling 3 minutes.
[b] Reagent remained colorless after boiling 3 minutes.
[c] Sucrose solution gave no precipitate of cuprous oxide when boiled with Fehling's solution.

Table II. Color Reaction of Phosphomolybdic Acid with Metals and Reducing Compounds

Reducing Substance	Color	Remarks
Mg (turnings)	Green, bluish green, then blue	Color changed very slowly
Al (powder)	None	Blue color produced when concd. H_2SO_4 added and mixture warmed
Zn (dust)	Blue	Color produced immediately
Fe (powder)	Green, bluish green, then blue	Color changed very slowly
Ni (granules)	Green, bluish green, then blue	Color changed very slowly
Sn (granules)	Green, bluish green, then blue	
Pb (granules)	Blue	Color developed slowly
Sb (granules)	Bluish green	Color developed slowly
Bi (granules)	None	Bluish green color produced when dilute H_2SO_4 added and mixture boiled
As (granules)	Bluish green	Color developed slowly
Cu (granules)	Blue	Color developed slowly
Hg	Bluish green	
Ferrous sulfate (0.1%)	Bluish green	
Ferrous ammonium sulfate	Bluish green	
Stannous chloride (0.1% in 0.1% HCl)	Blue	
Sodium bisulfite	Green, bluish green, then blue	Color changed very slowly; more rapidly if solution was boiled
Sodium thiosulfate	Green, bluish green, then blue	Color changed slowly; more rapidly if solution was warmed
Sodium hydrosulfite	Deep blue	
Potassium iodide	None	Bluish green color developed when solution was slightly warmed
Potassium ferrocyanide	Bluish green	Color changed to reddish brown when H_2SO_4 added
Hydroxylamine hydrochloride	None	Bluish green color developed when solution was boiled
Hydrazine hydrochloride	Green, bluish green, then blue	
Phenylhydrazine hydrochloride	Deep blue	
p-Aminophenol hydrochloride	Blue	
Hydroquinone	Blue	

PROCEDURE. *Color Reaction of Phosphomolybdic Acid and Molybdate with Sugars.* One milliliter of the sugar solution (5%) was added to the proper amount of the reagent. The whole was boiled for 3 minutes, the volume of the solution being kept unchanged by constantly adding water to it. Six common sugars were tested against phosphomolybdic acid, acidulated phosphomolybdic acid, ammonium molybdate, and acidulated ammonium molybdate. The results are tabulated in Table I.

Color Reaction of Phosphomolybdic Acid with Metals and Reducing Compounds. The metal or the solution of the reducing compounds (1 to 2 ml.) was added to 3 ml. of the test solution. The color reaction usually took place at room temperature; in only a few cases was warming or boiling necessary. The reducing compounds were 1% aqueous solutions, if not otherwise specified. The results are tabulated in Table II.

The phosphomolybdic acid did not give color reaction with formaldehyde, formic, lactic, and oxalic acids even when the solution was boiled.

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Separation of Catalysts from Hydrogenation Reaction Mixtures

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DURING the course of a research investigation, the problem of the removal of large quantities of hydrogenation catalysts, such as Raney nickel, platinum, or palladium, from the reduction medium was encountered. The usual methods involve filtration through paper by suction or use of the centrifuge. The former operation is by no means satisfactory, since the finely divided catalyst often passes into the filtrate, and, in addition, the pyrophoric nature of these metals produces sparking and charring of the filter paper. This becomes a definite fire hazard when large quantities of catalyst and inflammable solvent are handled. Removal of the catalyst by centrifugation often gives better results, but involves more manipulation, which may be deleterious to easily decomposed reduction products.

A procedure which has given good results but does not suffer from the deficiencies listed above, entails the use of a filter aid, such as Dicalite 4200, or its equivalent, spread in a layer about 1 cm. thick over filter paper seated in a Büchner funnel of appropriate size. This technique, which is by no means a new one in the industrial or analytical field, gives sparkling filtrates completely free of catalyst, and minimizes the possibility of ignition of the solvent or metal.

Test runs on various compounds have indicated that little if any material is adsorbed during this treatment, and the noble metal catalysts may be recovered without difficulty.

This procedure is advantageously modified in most cases by including the filter aid with the compound to be hydrogenated so that it is present during hydrogenation. This tends to give a better suspension of the catalyst and in many cases a better color of the finished product is obtained.

Instrument for Measuring Changes in Texture of Dehydrated Fish

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A NUMBER of mechanical devices for the measurement of tenderness of various kinds of food products such as peas and beefsteak have been described but none seems exactly suitable for dehydrated fish. The undesirable feature, inherent in an organoleptic method, is that the tester cannot accurately carry over from one testing period to another the standards for the degrees of texture evaluation. Thus any observations regarding the course of change occurring during an extended period of storage might be subject to considerable error. In order to eliminate the irregularities to which organoleptic tests are so susceptible and to be more directly applicable to the need, an instrument has been devised to record numerical values proportional to the changes in texture occurring in dehydrated fish during storage.

Figure 1. General View of Instrument

The new instrument consists essentially of a set of shearing plates or jaws, a supporting stand, a spring scale of 54.5-kg. (120-pounds) capacity, and a geared-down winch (Figure 1). The shearing jaws consist of 5 upper and 6 lower tool steel plates with square ground edges, 0.47 cm. ($^3/_{16}$ inch) thick by 3.75 cm. (1.5 inches) wide, by 12.5 cm. (5 inches) long for the upper and 10.94 cm. (4.375 inches) for the lower. These are so arranged that the upper plates nest between the lower ones with approximately 0.025-mm. (0.001-inch) clearance, so that a positive shearing action is effected. As may be seen in Figure 2 the sample compartment, which is 5.16 cm. ($2^1/_{16}$ inches) long by 2.5 cm. (1 inch) in diameter, is formed by cutting away a portion of the plates of the lower jaw and providing a shield on each side. The cut-away portion of the top jaw exactly coincides with that of the lower jaw when they are closed. Thus the sample compartment can be easily cleaned by raising the shields at the sides and brushing out the sample residue.

The sample to be tested is placed loosely and evenly in the compartment with the jaws open and the shields lowered into position. The upper jaw is then lowered until it rests on the sample, the spring scale is hooked to it, and a pulling force is applied to the scale by means of a cable fastened to the winch below. The force necessary to shear the sample is read directly in pounds from the dial. As the sample is sheared the upper jaw drops suddenly, thus releasing the pressure on the scale, and the maximum reading is registered by means of a friction hand. The scale is then unhooked, the shields are raised, and the sample residue is brushed out of the compartment into the waste can located directly below the jaws. The upper jaw is again raised, the shields are lowered into position, and the instrument is ready for another sample.

Preliminary tests indicated that to get uniform data the sample must be of uniform size and a uniform rehydration procedure must be followed in its preparation.

Ten grams of the dehydrated fish are allowed to rehydrate in 60 ml. of water at room temperature for 30 minutes. Temperature of rehydration as shown by Hamm, Butler, and Heerdt (*1*) is not critical, so that exact control of temperature during rehydration is not necessary. The reconstituted sample is then drained free of water on an inclined screen for 2 minutes and 10-gram samples of the reconstituted material are used for the measurements.

Table I. Change in Texture of Dehydrated Fish upon Storage as Indicated by Instrument Readings

	Original	After 14 Days	After 30 Days
	14.0	23.6	32.8
	14.5	24.6	38.8
	16.0	28.0	36.2
	16.0	24.0	35.4
	13.5	22.8	38.2
	17.5	25.8	35.4
Av.	15.2	24.8	36.1

Extensive tests with samples of various lots of dehydrated fish gave standard deviations of 1 pound for samples of the order of 10 pounds' toughness and 4 pounds for samples measuring 50 pounds. From these data it may be inferred that the average toughness value of 4 to 6 samples will be measured with a precision of less than ±5%. Table I is an example of the data obtained under actual operation.

Figure 2. Shearing Jaws

Organoleptic tests for texture changes made parallel with tests on the instrument have shown that the samples are placed in the same order in relation one to another but that small differences shown by the instrument are not always detected organoleptically.

This instrument is not designed to evaluate the quality of an unknown sample of dehydrated fish, but is of value in following the change in texture that occurs on storage. While no tests have been made on products other than dehydrated fish, it might be adapted for use with other such dehydrated products where alteration in texture during storage is a problem.

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A General Utility Laboratory Distillation Column

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MANY articles have been written on the design of laboratory distillation columns, but the construction details of a practical column, suitable for general distillation operations, are not easily available to those not familiar with this field. The column described in this paper is believed to embody most of the characteristics desirable for general utility. It is easily constructed of commonly available materials, suitable for most distillation operations over a wide temperature range, and free from mechanical or thermal strains. The essential feature is a glass heating jacket in which any desired distillation tube up to 45-mm. outside diameter may be inserted. There is also shown (Figure 1) a still head which is suitable for almost every type of distillation operation encountered in the laboratory. Its applications are discussed briefly.

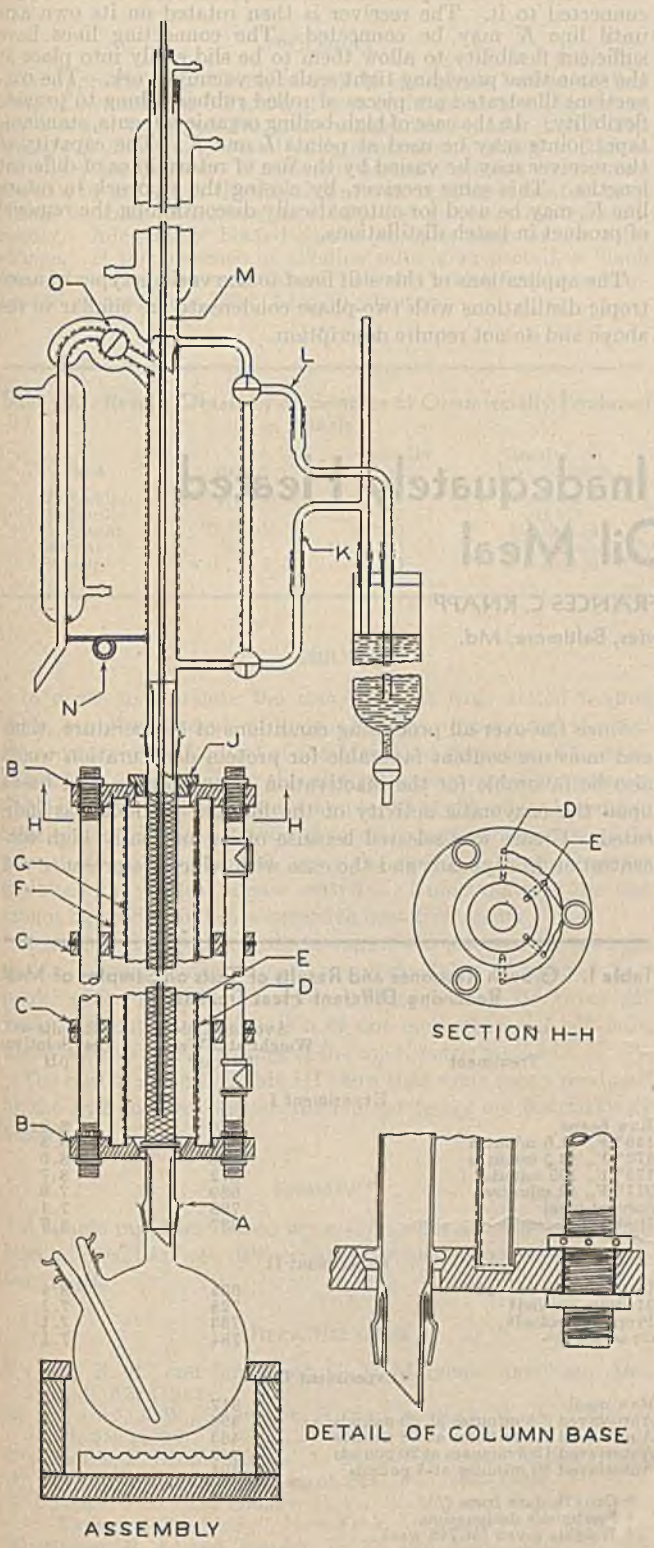

Figure 1. Diagram of Column

CONSTRUCTION

JACKET. The jacket, which is electrically heated, is made up of two concentric glass tubes, *G* 55 and *F* 70 mm. in outside diameter, held in place loosely by grooves cut in the two $^3/_4$-inch transite disks, *B*. These disks are bolted rigidly to a framework of three $^3/_8$-inch steel pipes, so that there is no mechanical strain on the glass. This arrangement allows both tubes to expand or contract independently of each other upon heating and cooling. The pipe framework is made rigid by means of two transite collars, *C*, fastened intermediate to the disks by setscrews. The inner glass tube is wound with two double-spiral, 20-ohm heating elements, *D*, so that the temperature of the upper and lower halves of the jacket may be adjusted independently. The heating elements are wound directly on the glass and held in place by moistened alundum cement. The ends of the elements are fastened securely by wire bands. The temperature of the jacket is measured by two thermocouples, *E*, placed inside the 55-mm. tube with the hot junctions located one fourth and three fourths of the distance along the jacket. The thermocouples are preferably strung as single strands running the length of the jacket. The electrical connections are made to the inner surfaces of the two end disks and the wires strung through the pipe framework.

DISTILLATION TUBES. The distillation tubes, which may be as large as 45 mm. in outside diameter, are inserted through the opening in the upper transite disk and rest upon the tapered opening of the bottom disk. The tubes smaller than 45 mm. in outside diameter are centered in the upper disk by means of split transite collars, *J*, and are provided with a ring of glass at the lower end if they are smaller than the bottom opening. (As an alternative arrangement the top of the distillation tube may be provided with a ring of glass which rests on the upper opening.) This arrangement, intended for use with standard-taper joints, allows the tube assembly to rise and pivot about the top when the bottom accessories are being attached. Breakage is thus prevented when the pieces are not correctly aligned. The distillation flasks are joined to the column by suspending them in a clamp attached at *A*, so as to raise the tube off the tapered opening in the bottom disk. This allows the tube to seat itself in the flask by its own weight.

STILL HEAD. A still head, which has been found suitable for many operations, is constructed so that it may be inserted in the top of the distillation tube without clamping, thus facilitating the assembling of the bottom accessories. The head is constructed of a straight tube 25 mm. in outside diameter and is provided with vapor, *O*, and liquid, *L*, sampling lines and also a line, *K*, for returning liquid to the column. The inner tube of sample condenser in the vapor line should be constructed of 8-mm. tubing in order to prevent a liquid leg from forming in the condenser. The vapor line, together with its stopcock, and that portion of the head below the sampling lines are provided with a 30-ohm heating element. The windings on the vapor line and stopcock are wound over asbestos paper and coated with moistened alundum cement. The rest of the windings are wound directly on the glass. A thermocouple is wound around the barrel of the stopcock to measure the temperature at that point. A capillary thermocouple well, *M*, extending through the still head and, in the case of a packed column, down through the packing, may be used to measure the temperature at all points in the column.

DISCUSSION

The column described above may be used for practically all types of distillation operations which are conducted above

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room temperature and at pressures varying from a few millimeters of mercury absolute to several pounds per square inch gage. It is suitable for the analogous operations of absorption and extraction and may also be used as a reaction tube. In the latter case, the reaction tube should be provided with an additional heating element. While the design of this column is best suited for operations above room temperature, it may also be used for low-temperature work where it is necessary to observe the column action. In this operation, the cooling fluid would be circulated in the annular space between the 55- and 70-mm. jacket tubes. If the fluid was introduced several inches below the upper disk, only the lower end of the jacket would have to be sealed by a gasket.

The still head shown in the figure is a composite of several types used by the authors. The thermocouple well extending down through the packing is useful where the temperature is a criterion of the product compositions. While the thermocouple well will decrease the efficiency of the packing, this is in many cases compensated for by the gain in operability of the column. It enables the optimum reflux ratio to be set with a minimum of trial and error and the future course of batch distillations to be predicted without frequent readings of the temperature.

The vapor sampling line, O, is used where it is necessary to maintain a high reflux ratio and low holdup, as in the case of analytical distillations. Reflux ratios of 20/1 (O/P) are readily and accurately obtained by adjusting the stopcock and then applying a slight pressure to the column (or vacuum to the receiver). The take-off rate is closely proportional to the square root of the pressure applied. Other advantages of vapor sampling are that it minimizes contamination of the sample by the stopcock grease and allows accurate sampling of distillates which give two liquid phases upon condensation.

A simple method of attaching accessories to the still head is illustrated in the figure for operation at total reflux with product holdup.

The receiver is suspended in a clamp, so that line L may be connected to it. The receiver is then rotated on its own axis until line K may be connected. The connecting lines have sufficient flexibility to allow them to be slid easily into place at the same time providing tight seals for vacuum work. The connections illustrated are pieces of rolled rubber tubing to provide flexibility. In the case of high-boiling organic solvents, standard-taper joints may be used at points L and K. The capacity of the receiver may be varied by the use of return lines of different lengths. This same receiver, by closing the stopcock in return line K, may be used for automatically discontinuing the removal of product in batch distillations.

The applications of this still head to the various types of azeotropic distillations with two-phase condensate are similar to the above and do not require description.

Method for Detecting Inadequately Heated Soybean Oil Meal

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Editor's Note. Since receipt of this paper, a subcommittee of the Animal Nutrition Committee of the National Research Council has been appointed to study tests which might be applied to soybean oil meals to indicate the degree of heat treatment and to correlate it with biological efficiency. The committee is carrying on collaborative work with additional samples in order to check further the validity of the urease test.

THE high nutritive value of soybean oil meal for poultry and swine depends considerably upon the heat treatment used in its preparation. Adequate heat treatment improves the biological value of the proteins (*2*, *3*, *4*) and simultaneously inactivates the enzymes present (*6*). The enzyme lipoxidase if left active in the meal could readily cause the destruction of vitamin A or its precursors with which it comes in contact in the digestive tract of animals. The contemplated use of urea in feed mixtures for ruminants makes it important that the soybean oil meal used in such mixtures be heated sufficiently to inactivate urease. Mixtures of inadequately heated soybean meals or raw soybeans and urea develop the highly characteristic odor of ammonia and, hence, become unpalatable.

It has been reported by Bird and co-workers (*1*) that commercially produced meals differ markedly in their nutritive values when used as the principal source of protein in the chick ration. Some of the poor results obtained were attributed to the use of insufficiently heated meals. With the enormously increased production of soybeans and their subsequent conversion into meal by plants having no previous experience with this commodity, the need for a rapid test for determining adequacy of heat treatment is apparent.

Since the over-all processing conditions of temperature, time, and moisture content favorable for protein denaturation would also be favorable for the inactivation of enzymes, a test based upon the enzymatic activity of the finished product was indicated. Urease was selected because of its unusually high concentration in soybeans and the ease with which its presence can

Table I. Growth Response and Results of Tests on Samples of Meal Receiving Different Heat Treatments

Treatment	Average Chick Weight at 9 Weeks, Grams[a]	Results on Test Solution, pH
Experiment I		
Raw beans	476	8.9
143° F., 11.6 minutes	528	8.8
173° F., 16.5 minutes	586	8.6
175° F., 105 minutes	572	8.7
217° F., 42 minutes	669	7.6
Solvent meal	798	7.1
Hydraulic meal B	637	8.9
Experiment II		
Insufficiently cooked[b]	664	8.4
Medium cooked[b]	728	7.1
Properly cooked[b]	733	7.1
Overcooked[b]	784	7.1
Experiment III[c]		
Raw meal	317	8.6
Autoclaved 2.5 minutes at 20 pounds	494	7.1
Autoclaved 7.5 minutes at 20 pounds	483	7.1
Autoclaved 12.5 minutes at 20 pounds	443	7.1
Autoclaved 60 minutes at 5 pounds	494	7.1

[a] Growth data from (*1*).
[b] Producer's designation.
[c] Weights given for 7th week.

Table II. Precision of Test

Date	Results on Test Solution, pH
May 27	7.59
August 2	7.68
August 9	7.72
August 10	7.55
August 11	7.59

be detected. The following test based upon Sumner's (*5*) qualitative test for this enzyme was devised.

PROCEDURE

To approximately 10 ml. of 0.05 *M* phosphate buffer solution of pH 7.0 containing 0.3 gram of urea and 2 drops of 0.1% phenol red solution is added 0.2 gram of the meal under test. The mixture is allowed to stand with occasional shaking at 25° to 30° C. for 30 minutes. If sufficient urease is present to cause an increase in the pH of the solution of one unit as indicated by a change in color to deep red, the meal has not been heated sufficiently. Adequately heated meals produce little or no color change. If the presence of alkaline salts is suspected, a blank should be run, using a sample of the meal which has been inactivated by heating at 135° C. for 30 minutes.

Table III. Results Obtained on Samples of Commercially Produced Meals

Type	Brand	Samples Tested	Results on Test Solution
Hydraulic	A	7	pH 8.3
Hydraulic	B	12	pH 8.5
Hydraulic	C, D, E	16	No change
Solvent	H, L	5	No change
Expeller	A, F, G, I, J	45	No change

RESULTS

In order to correlate the chemical test with actual feeding value, samples of meals of known history were secured through the courtesy of H. R. Bird of the Maryland Experiment Station. The results obtained using this test on those samples are given in Table I. The readings are given in terms of pH values instead of color change because of the greater accuracy obtainable. In general, those meals which gave the poorest growth response exhibited the highest urease activity. Unfortunately, the test cannot be used to indicate excessive heat treatment.

A sample giving intermediate values was selected for periodic testing in order to determine the reproducibility of results. The results given in Table II were obtained with a Beckman pH meter and indicate a variation of not more than 0.2 pH unit, which is well within the range of the colorimetric method.

The results given in Table III show that some meals produced by the hydraulic process on the market today are insufficiently heated.

SUMMARY

A simple rapid test based upon the urease activity of the soybean oil meal has been devised to detect inadequately heated soybean meals.

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Dropping Funnel

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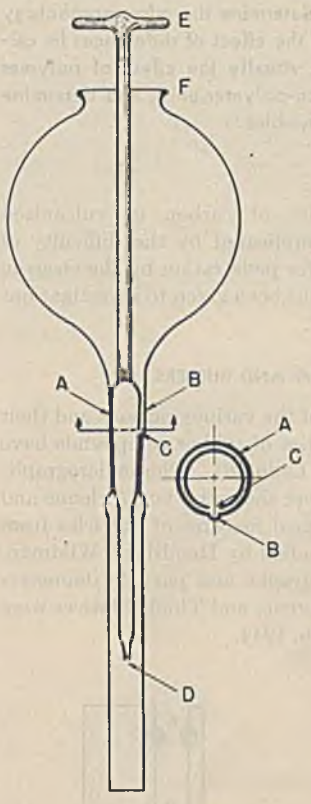
IN WORK for which a steam-jacketed dropping funnel is required the ordinary type is unsatisfactory because of difficulty in manipulating the stopcock to give a regulated flow of liquid from the funnel. It is also unsatisfactory where the liquid contained in the funnel must not dissolve stopcock grease.



The figure shows a dropping funnel which does not possess these difficulties. This funnel contains an internal ground joint, *A*. The liquid in the bulb flows down a narrow groove, *B*, in the outer wall and through a 2-mm. hole, *C*, in the hollow center plug (see enlarged cross-sectional view). This tapers down to a dropping point, *D*, which shows the rate of flow through opening *C*. On the upper lip of the top of the funnel is a small point of glass, *F*, and the handle, *E*, is placed in such a position that *E* and *F* are in the vertical plane with *B* and *C* when the hole is in the open position.

The entire funnel and ground joint can be placed in a steam or hot water bath, the ground seat needs no lubricant, for the contained liquid will so act, and if any liquid leaks through the joint it can drop only into the reaction flask.

ACKNOWLEDGMENT

The author wishes to thank Ray Newberry, university glassblower, for his work in constructing this apparatus.

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Collective Index of Analytical Edition—Progress Report

Work on the fifteen-year cumulative index to the Analytical Edition of Industrial and Engineering Chemistry, first announced in the July issue, has gone forward steadily, and it is now planned to send the index to the printer in November, so that distribution can be begun early in 1945.

The index will form a book about the same size as an issue of the Analytical Edition, and will be printed on the same paper. It is being prepared by Charles L. Bernier, Associate Editor of *Chemical Abstracts*.

In the November 15 issue of the Analytical Edition will appear a definite announcement of price and date of issue, with full instructions regarding placing of orders, for the benefit of those who have not yet done this.

Electron Microscope Studies of Colloidal Carbon in Vulcanized Rubber

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Columbian Carbon Company, Research Laboratories, Brooklyn, N. Y.

New techniques are described for electron microscopy studies of colloidal carbon in vulcanized natural and synthetic rubber, by which it is hoped to make it possible to determine the micromorphology of carbon-reinforced rubbers, assess the effect of differences in carbon fineness and structure, evaluate visually the effect of polymer differences upon the ultimate carbon-polymer units, and determine the effect of processing and other variables.

ELECTRON microscope studies of carbon in vulcanized rubber have always been complicated by the difficulty of preparing specimens thin enough for penetration by the electron beam. The main attention has thus been given to investigations of carbons and rubbers separately.

WORK ON CARBONS AND RUBBERS

Discussions of the particle size of the various carbons and their correlation to the physical properties of rubber compounds have been published by Wiegand and Ladd (*6*). Photomicrographs of natural and Perbunan latices were shown by von Ardenne and Beischer (*1*) in 1940. Morphological features of particles from latices of 16 plant species were studied by Hendricks, Wildman, and McMurdie (*3*). Photomicrographs and particle diameters for natural, Buna S, Buna N, neoprene, and Thiokol latices were published by Wiegand (*5*) in March, 1944.

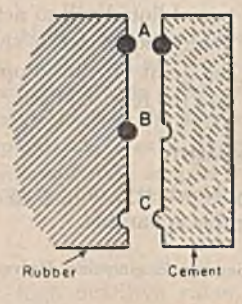

Figure 1

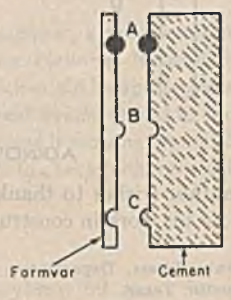

Figure 2

Other studies have been made on cements and fibers. Von Ardenne prepared films of rubber by spreading a thin film of latex on a glass slide, breaking the slide, and stretching the film. He also cast films from a solution of rubber in benzene (*1*). Studies on vulcanized and unvulcanized rubber have been carried out by Hall and co-workers (*2*) by allowing films cast from a cement to break into fibers and examining these fibers in the electron microscope.

CONTEMPORARY WORK ON COMPOUNDS. The earliest pictures of vulcanized, carbon-reinforced rubber were shown by von Ardenne (*1*) who prepared the specimen by crushing a sample of vulcanized rubber cooled by liquid air, and then choosing the finest fragment by means of a light microscope. Prebus (*4*) prepared rubber specimens by cutting a section of cable insulation by means of an abrasive wheel. The number of fragments suitable for electron microscope investigation made by either of these two methods is extremely small and therefore other means of preparation were desirable.

DEVELOPMENT OF NEW METHODS

As a first attempt in investigating carbon-rubber specimens, studies were made on uncured tread stock compounds made into cements from which thin films were cast. The rubber films were supported on collodion to prevent their breaking into fibers. These studies gave evidence of the ability of "structure" carbons to survive the shearing stress involved in milling in rubber (*5*). However, it was felt that dissolving the rubber compound, and then casting a film, changed the dispersion from that of the milled stock. Consequently other methods were sought. Three methods have been developed:

1. RUB OUT TECHNIQUE. In this method, a Formvar film is first obtained on a glass microscope slide. A small quantity of uncured stock is then rubbed out on the Formvar by strokes of a spatula or the edge of a glass slide. A 200-mesh screen is then cemented to the rubber smear by means of Ambroid around its edge. The Formvar film is teased free and floated on a water surface from which it is taken and allowed to dry.

The specimens can be examined in the electron microscope, given a dry heat cure, and photographed again.

The disadvantages in this method are: Method of smearing is such that streaking in one direction is produced; and dry heat cure is different from that employed in pressure molds.

2. REPLICA METHOD. This involves cracking a rubber block and obtaining a Formvar replica of the broken surface. The appearance of the carbon in the broken surface is analogous to that of stones in a broken concrete block.

A cured rebound block (2 × 1 × 1 inch) made of standard tread stock is first frozen in an acetone–dry ice bath, then placed in a vise and cracked into two pieces. First attempts to obtain

CRD-1

Figure 3. P-33 in GR-S (X 5000)

a replica of the broken surface involved putting a Formvar film directly on the rubber. A suitable film for the electron microscope could not be stripped off, however. In order to overcome this difficulty, molten medium DeKhotinsky cement was poured on the surface (polystyrene could also be used). This was stripped off when hard and a drop of 2% Formvar solution in ethylene dichloride placed on the impression. After the film had hardened, the cement was dissolved in Solox. The Formvar replica obtained was then photographed.

The interpretation of the resultant photomicrograph requires a series of prints made at varying exposures. Three types of densities are representative of carbon particles in the original block.

Particle A. The original broken surface will have carbon particles protruding and holes where particles remaining in the other half of the block have been ripped out.

A protruding particle (*A*, Figure 1) may be ripped out by the cement. This will be carried directly into the Formvar film (*A*, Figure 2) when the cement is dissolved and will be a black circle on the print.

Particle B. A protruding particle (*B*, Figure 1) may remain in the rubber block and will be represented by a hole in the cement. This will give rise to a pimple on the Formvar film, and in the print will give a black circle lighter in density than that corresponding to particle *A*.

Particle C. A hole (*C*, Figure 1) in the block will be carried over to the Formvar as a hole. This will appear in the print as a white circle.

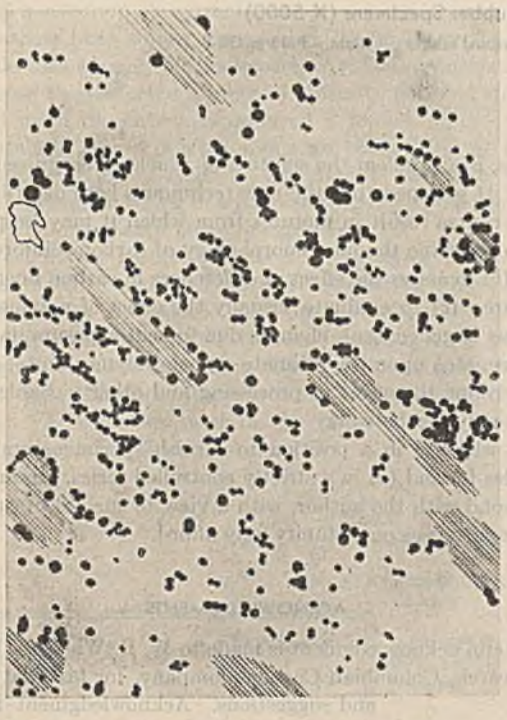

Figure 4. Dispersion of P-33 in GR-S Block

A photomicrograph of a replica from a block of P-33 in GR-S is shown in Figure 3, overexposed to bring out the white circles (particle *C*). Bits of rubber pulled out by the cement are also evident.

In Figure 4 is a map made from the various prints, showing the actual dispersion of P-33 in the GR-S block.

3. Vulcanizing Method. This method has been developed more than the preceding two, because of its greater efficiency. In principle it consists of pressing out the uncured stock to a thin film and then vulcanizing it.

The rubber is pressed out between two specially prepared disks 0.25 inch in diameter (Figure 5). Disk *A* is pressed out of $^1/_{16}$ inch steel plate with a die shaped to give a crown. Filing the one side flat gives the shape shown in Figure 5. Disk *B* is flat and pressed out of $^1/_{16}$ inch aluminum. The inner faces of both *A* and *B* are coated with collodion, which serves a twofold purpose: it fills any holes in the metallic surfaces and thus allows slippage of the rubber as the pressure is applied, and it enables the extremely thin film to be removed from the mold. The piece of rubber tread stock placed between the two disks is less than $^1/_{32}$ inch in diameter.

Figure 5. Disks

Three sets of disks with rubber between them are placed between two 6-inch square flat mold plates. In Figure 6 one of the sets has been left separated to show the piece of rubber in position. The mold is then placed in a Carver press, a pressure of 1000 to 4000 pounds per square inch is applied, and the specimens are cured.

After curing is complete, the disks are separated and placed in amyl acetate. This dissolves the collodion and the pieces of thin film are teased free, allowed to remain in the amyl acetate for several days, then picked up on 200-mesh screens, and photographed in the electron microscope.

PHOTOMICROGRAPHS

Photomicrographs of specimens of vulcanized rubber prepared by the above method are shown in Figure 7. The compounds were as follows:

Micronex W-6 in GR-S	
GR-S	100.0
Micronex W-6 (EPC)	50.0
Zinc oxide	3.0
Bardol	7.5
Benzothiazyl sulfenamide	1.2
Sulfur	1.8
Micronex W-6 in Natural Rubber	
Smoked sheets	100.0
Micronex W-6 (EPC)	50.0
Zinc oxide	3.0
Stearic acid	4.0
Pine tar	2.0
BLE	1.5
Sulfur	2.7
MBT	0.9
P-33 in GR-S	
GR-S	100.0
P-33 (FT)	50.0
Zinc oxide	5.0
Bardol	4.5
Pine tar	3.0
Sulfur	1.8
Benzothiazyl sulfenamide	1.0

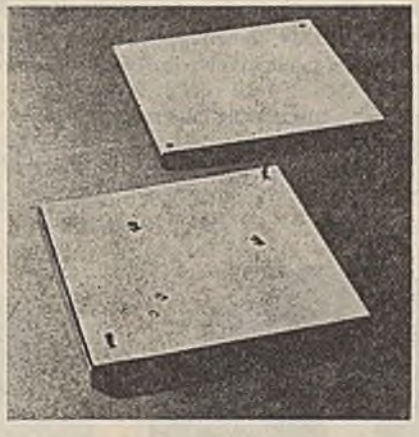

Figure 6. Mold Plates

Several tests were made to check on whether the carbon was being squeezed out of the rubber onto the collodion and might become insoluble in amyl acetate during curing. One test consisted of taking stereoscopic pictures. These reveal the carbon to be inside the rubber film. Figure 8 presents a pair of stereopictures, which show interesting tears. The density at *A* indicates a thickness of rubber equal to that of the P-33 particle. The serrated edge at *B* matches the carbon particles at *C*. The

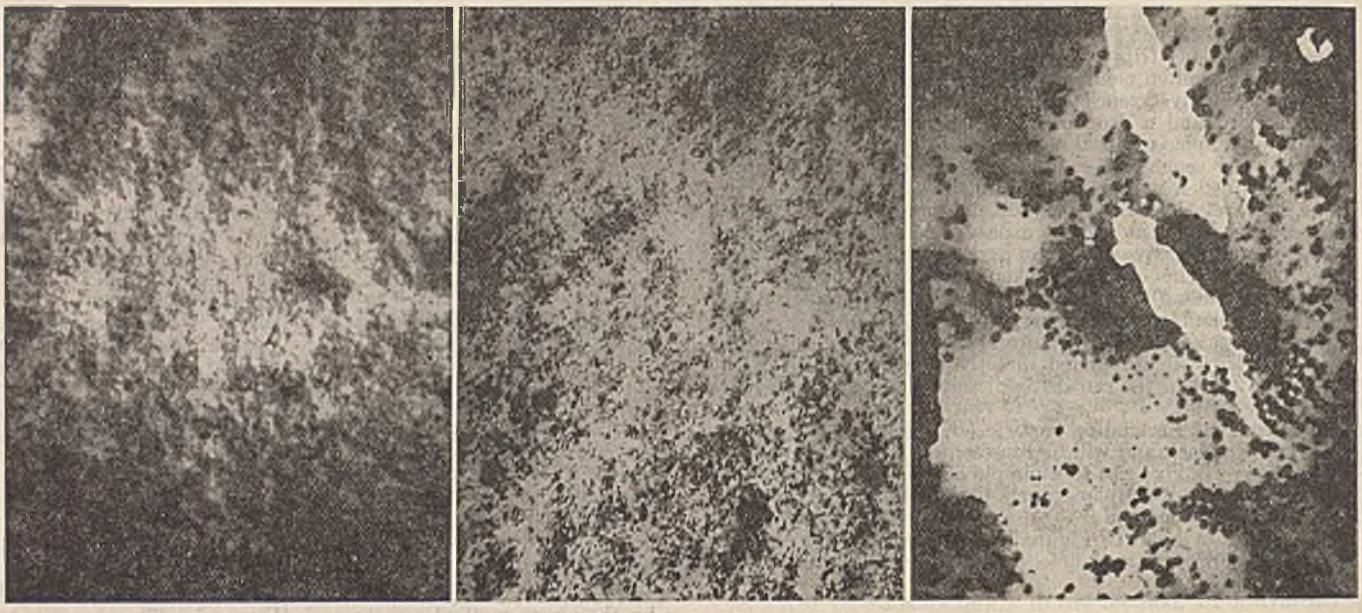

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Figure 7. Photomicrographs of Vulcanized Rubber Specimens (X 5000)

Left. Micronex W-6 in GR-S Center. Micronex W-6 in natural rubber Right. P-33 in GR-S

radius of curvature has increased, indicating a contraction of the rubber after tearing. The weakness of the bond between the P-33 particle and the rubber is also shown by the cleanness of the break.

DISCUSSION OF METHODS

The rub out technique, because of its directional effect, is poorest of the three methods.

The vulcanizing method is the most successful of the three, and gives the best pictures. Some distortion may be present due to the high pressures used.

The replica method involves no distortion of the rubber block. Its disadvantage lies in difficulty of interpretation of the pictures and poor definition in the case of replicas of the fine carbons.

Studies now being made involve use of the last two methods.

DISCUSSION OF RESULTS

The definitive analysis of the disposition of reinforcing carbon particles in natural, and even more importantly in synthetic rubbers, is a problem the solution of which is recognized as cardinal. It is hoped that the new techniques here described may in due course result in pictures from which it may be possible: (*a*) to determine the micromorphology of carbon-reinforced rubbers; (*b*) to assess the effect of differences in carbon fineness and structure; (*c*) to evaluate visually the effect of polymer differences, as in gel content, changes due to heat exposure, latex particle size, etc., upon the ultimate carbon-polymer units; and (*d*) to determine the effect of processing and other variables in the carbon-polymer network.

Any who are in a position to furnish specimens embodying variables (*c*) and (*d*) in a strictly controlled series, are invited to correspond with the author, with a view to such electron microscopic analysis as opportunity may afford.

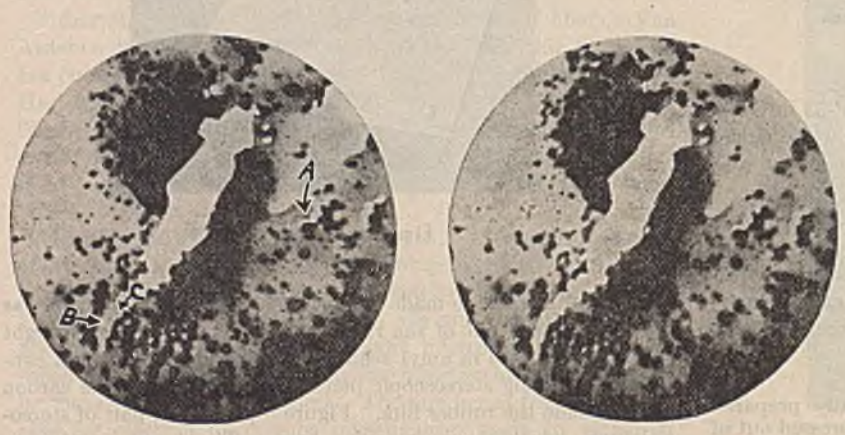

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Figure 8. Stereoscopic Pictures of P-33 in GR-S

ACKNOWLEDGMENTS

Grateful acknowledgment is made to W. B. Wiegand, Director of Research, Columbian Carbon Company, for his kind interest and suggestions. Acknowledgment is made to E. R. Gilliland, Assistant Rubber Director, for permission to publish at this time.

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Device for Projecting an Image of a Reading Scale

CLIFTON TUTTLE AND F. M. BROWN, Kodak Research Laboratories, Rochester, N. Y.

SINCE the optical system now in use in these laboratories to facilitate the reading of microchemical balances has proved satisfactory, the authors believe a description might be of value to others engaged in similar work.

For routine analyses, a number of Kuhlmann microchemical balances are used, in which the pointer passes across a white scale with black line indexes at 0.2-mm. intervals. Deflection of the pointer is estimated to a tenth of one of these divisions—an operation obviously impossible for the unaided eye. Ordinarily a monocular telescope magnifier giving 5× or 6× magnification is directed at the scale. Operators who make many daily readings in this manner suffer considerable eyestrain and fatigue in making precise observations and would therefore find desirable a projected scale image that could easily be observed with both eyes.

It appears impossible to provide sufficient light on the conventional white scale to make possible its projection with adequate brightness and sufficient magnification. A light source of wattage high enough to provide sufficient light cannot be used inside or even in proximity to the balance case because of the danger of setting up convection air currents.

The authors have solved the problem by replacing the white ivory scale with a highly efficient specular reflecting surface. For this purpose they selected a piece of optically polished stainless steel and upon its surface reproduced a replica of the microbalance scale by means of a technique worked out for the photographic reproduction of reticles. Mechanical engraving of the lines and subsequent filling in with black pigment would serve as well, but would probably be more expensive.

The steel mirror surface reflects about 80% of the incident light, most of which is utilized, instead of scattering it inefficiently as does the matte white scale. The optical system actually employed in directing light to the scale and receiving it for projecting a magnified scale image might well be of various forms and dimensions, depending upon the particular apparatus to which it is applied. The system used in the microbalances is shown diagrammatically in Figures 1 and 2.

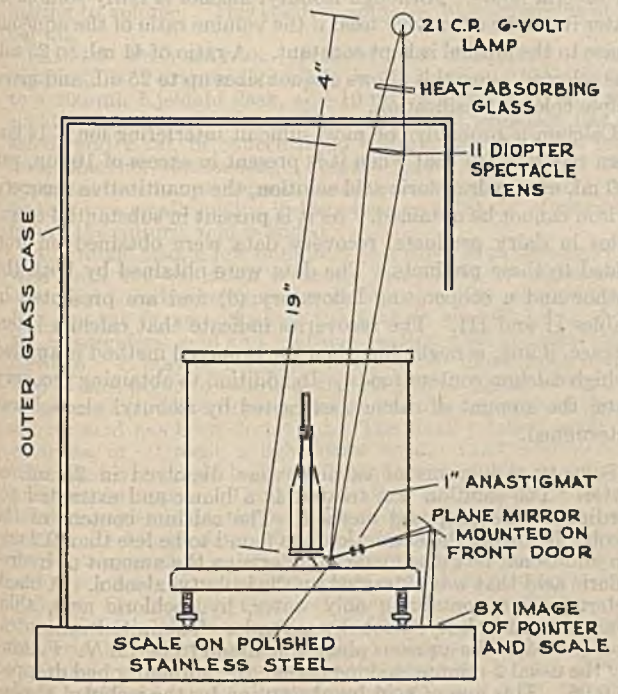

Figure 1. Microbalance with Scale Projecting System

In use, the balances are set up inside glass cabinets that protect the instruments from air currents and mechanical injury. The lamp in a housing is placed outside this outer cabinet. The filament of the lamp is imaged at about 5× magnification slightly above the stainless steel mirror. This slightly out-of-focus filament image produces acceptably uniform illumination on the mirror. The scale reflects the image into a 2.5-cm. (1-inch) focal length objective which projects the scale image and the image of the shadow of the pointer onto a white screen at a magnification of about 8×. For convenience, the image is directed downward by means of a small plane mirror, so that it falls on the bench in front of the instrument in a position which can be conveniently seen by the operator. The image is bright enough to be clearly visible in a well-lighted room. The illumination level is about 20 foot-candles.

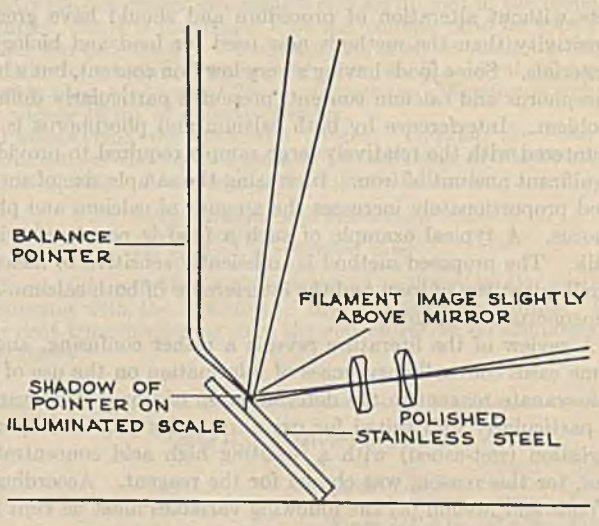

Figure 2. Side View of Pointer Scale, and Projection Objective

Since it is important to avoid heat within the balance case, it is desirable to determine how much energy is absorbed inside the case. The energy radiated by the lamp and accepted by the first lens amounts to about 0.2 watt, 80% of which is absorbed by the heat-absorbing glass (Corning Aklo). About 0.04 watt is the amount of energy reaching the balance scale. Almost 30% of this energy is reflected by the mirror surface through the lens and about 70%, or less than 0.03 watt, goes to heat the instrument. This is less than the radiant energy entering the case from other light sources in the room. Prolonged trial shows no heating effect upon the delicate mechanism of the balances.

COMMUNICATION 979 from the Kodak Research Laboratories.

Spectrographic Boron Steel Standards

The National Bureau of Standards, Washington, D. C., is prepared to furnish six samples of boron steels in rod form for spectrographic standards.

The standard samples are cylindrical rods $^{7}/_{32}$ inch in diameter and 4 inches long. The 4-inch rod may be cut at the center, giving two rods each 2 inches long for use as self-electrodes. The price per sample is $3.00.

No.	Kind	Total Boron, %
425	Mn-Ni-Cr (N.E. 9450)	0.0006
426	Cr-Mo (SAE 4150)	0.0011
427	Cr-Mo (SAE 4150)	0.0027
428	Mn-Cr	0.0059
429	Ni-Cr-B	0.0091
430	Ni-Cr-B	0.019

Determination of Iron in Food Products

JOHN B. THOMPSON

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A modified thiocyanate procedure in which the color complex is extracted with isobutyl alcohol is described. Data on recoveries of added iron to a wide variety of foods are given. Recoveries of better than ±0.5 p.p.m. are possible.

IRON content is an important factor in the nutritional evaluation of foods. As a trace contaminant promoting oxidative and metallic flavors, rancidity, and vitamin instability it also plays an important role. A satisfactory method for the determination of iron should be adaptable to a wide variety of products without alteration of procedure and should have greater sensitivity than the methods now used for food and biological materials. Some foods having a very low iron content, but a high phosphorus and calcium content, present a particularly difficult problem. Interference by both calcium and phosphorus is encountered with the relatively large sample required to provide a significant amount of iron. Increasing the sample size of such a food proportionately increases the amount of calcium and phosphorus. A typical example of such a food is powdered whole milk. The proposed method is sufficiently sensitive to measure small quantities of iron, and the interference of both calcium and phosphorus is negligible.

A review of the literature reveals a rather confusing, and in some cases contradictory, mass of information on the use of the thiocyanate reagent in the determination of iron. Thiocyanate is particularly well suited for use on samples prepared by acid digestion (wet-ashed) with a resulting high acid concentration and, for this reason, was chosen for the reagent. According to Woods and Mellon (*8*) the following variables must be kept reasonably constant: (1) amount and kind of acid, (2) excess quantity of oxidizing agent, (3) time of standing, (4) presence and amount of certain interfering ions, and (5) dielectric constant of the solvent.

Pyrophosphates resulting from dry-ashing interfere with the determination by complexing the iron (*8*). This interference can be eliminated by digesting the sample in sulfuric, nitric, and perchloric acids. Jackson (*4*) used acid digestion satisfactorily, and has shown that excellent recoveries can be obtained. Acid digestion is particularly advantageous where copper is also to be determined, as both determinations can be made from a single sample preparation.

Most foods may be readily ashed by digestion with sulfuric, nitric, and perchloric acids. However, a few products of high fat content do not lend themselves readily to complete oxidation. Dairy products are particularly difficult to digest, and at best, their sample preparation is a tedious, time-consuming procedure. Thirty per cent hydrogen peroxide has been used to excellent advantage in the oxidation of both high fat and carbohydrate products (*5*). The alternate ashing procedure described under the sample preparation has reduced the time for the preparation of a 5-gram sample of powdered whole milk to less than one half of that required when peroxide is not used.

Hallinan (*3*) has shown that a high concentration of hydrochloric acid is exceptionally well suited for the development of the ferric thiocyanate complex and that a high concentration of potassium thiocyanate gives the maximum color development in the presence of a minimum concentration of iron. A concentration of 1.5 *N* hydrochloric acid and 0.5 *N* potassium thiocyanate in the presence of potassium persulfate as an oxidizing agent gives excellent color development and stability. Potassium persulfate may be used satisfactorily as an oxidizing agent both to ensure that iron remains as the ferric ion and to stabilize the color complex in aqueous solution. Although potassium persulfate has been reported to develop a yellow color in hydrochloric acid solution (*8*), this has not been noted in this study. The comparative stability of the ferric thiocyanate complex with and without added persulfate is shown in Table I.

Table I. Comparative Stability of Ferric Thiocyanate Complex in the Aqueous Phase

Time of Standing	Transmission: No potassium persulfate added	Transmission: Potassium persulfate added (1 ml. of 2% solution)
Min.	%	%
0 (initial reading)	50.2	50.4
5	51.0	50.3
10	52.2	50.4
15	52.8	50.3
20	53.8	50.4
35	55.5	50.5
45	56.8	50.9
60	59.4	51.0

Isobutyl alcohol appears to be an ideal solvent for concentrating and intensifying the color, and a satisfactory means of eliminating the interference of calcium. The ferric thiocyanate complex extracted from an acidified aqueous solution with isobutyl alcohol has a maximum absorption at 485 millimicrons, obeys Beer's law (Figure 1), and is free from the variations which occur when isoamyl alcohol is used (*2*). The point of maximum absorption was determined using a Coleman Universal spectrophotometer and checked with a Beckman quartz spectrophotometer. Winsor (*7*) has shown in his study of the intensity and stability of the complex that the dielectric constant is a satisfactory criterion for the dissociation of the solute. Isobutyl alcohol has a relatively low dielectric constant (International Critical Tables value of 18.7) and the resulting solution of the complex is stable for several hours. Although isobutyl alcohol is fairly soluble in water it can be effectively used if the volume ratio of the aqueous phase to the alcohol is kept constant. A ratio of 41 ml. to 25 ml. was selected, since this allows aliquot sizes up to 25 ml. and gives ample color intensification.

Calcium is probably the most difficult interfering ion. It has been reported (*2*) that when it is present in excess of 10 mg. per 100 ml. of a hydrochloric acid solution, the quantitative recovery of iron cannot be obtained. As it is present in substantial quantities in dairy products, recovery data were obtained on iron added to these products. The data were obtained by both the author and a cooperating laboratory (*6*) and are presented in Tables II and III. The recoveries indicate that calcium interference, if any, is negligible when the proposed method is applied to high calcium content foods. In addition to obtaining recovery data, the amount of calcium extracted by isobutyl alcohol was determined.

Seventy milligrams of calcium were dissolved in 25 ml. of water. The solution was treated as a blank and extracted according to the proposed method. The calcium content of the alcohol following the extraction was found to be less than 0.2 mg. An additional test was made to determine the amount of hydrochloric acid that was extracted by the isobutyl alcohol. A blank determination containing only water, hydrochloric acid, thiocyanate, and isobutyl alcohol was made. The initial concentration of acid in the aqueous phase was found to be 1.3 *N*. Following the usual 2-minute shaking period, the normality had dropped to 0.96. This loss of acid by absorption by the isobutyl alcohol increases the normality of the solvent to approximately 0.55 *N*.

The presence of a relatively high concentration of hydrochloric acid in the isobutyl alcohol undoubtedly aids in the stabilization of the ferric thiocyanate complex.

The stability of the ferric thiocyanate complex has been checked on several determinations by reading the per cent transmissions immediately and then again over periods ranging up to 4 hours. In no cases were increases in transmittancies noted. One sample with an initial reading of 23% was read again at the end of 36 hours. The transmission was found to be 26% or an increase of only 3%. However, recovered isobutyl alcohol previously used for either thiamine or iron determinations resulted in rapid fading and hence could not be used satisfactorily.

A single distillation from an all-glass Pyrex still frees isobutyl alcohol from its initial iron content and, inasmuch as it is a standard solvent for thiamine assays, it is readily available in food laboratories doing vitamin analyses.

METHOD APPLICATION

Apparatus. A spectrophotometer or photoelectric colorimeter (a Coleman Universal spectrophotometer was used by the author). Readings are made at 485 millimicrons, the point of maximum absorption.

Pyrex glassware. All glassware is cleaned with concentrated nitric acid, rinsed with distilled water, and finally rinsed several times with redistilled water.

Reagents. Sulfuric acid, concentrated, reagent grade.

Nitric acid, concentrated, reagent grade, redistilled from Pyrex.

Perchloric acid, double-vacuum-distilled 72% perchloric acid (may be obtained from G. Frederick Smith Chemical Co., Columbus, Ohio).

Hydrogen peroxide, 30%, without added stabilizer.

Redistilled water, distilled water redistilled from Pyrex.

Hydrochloric acid, concentrated, reagent grade.

Potassium persulfate, reagent grade, 2% solution in redistilled water, prepared fresh every few days and stored in a refrigerator.

Potassium thiocyanate, reagent grade, 20% solution in redistilled water prepared frequently and stored in a refrigerator.

Isobutyl alcohol, b.p. 106–107° C., redistilled from Pyrex.

Standard Iron Solutions. Stock Solution. Weigh exactly 1.0000 gram of iron wire into a dry, iron-free beaker. Dissolve in 20% hydrochloric acid to which 1 to 2 ml. of concentrated nitric acid have been added. Carefully evaporate to dryness and dissolve in the minimum amount of hydrochloric acid. Transfer quantitatively to a 1000-ml. volumetric flask and dilute to volume. This stock solution contains 1 mg. of iron per ml.

Working Standard. Dilute 10 ml. of the stock solution to 1000 ml., adding a few drops of bromine water just prior to adjusting to volume. This solution contains 10 micrograms of iron per ml.

Sample Preparation. Transfer an accurately weighed sample (3 to 5 grams, depending on the suspected iron content) to a 300-ml. Kjeldahl flask, add 10 ml. of nitric acid, and warm slightly to start oxidation. When the initial oxidation has subsided, add 2 ml. of concentrated sulfuric acid and boil gently until charring commences. Prepare liquid samples by taking appropriate volumes, depending on the iron content, and concentrating to a small volume in the presence of 10 ml. of nitric acid before the sulfuric acid is added.

Add nitric acid, a few milliliters at a time, or preferably, dropwise until the oxidation is nearly completed as evidenced by only slight darkening upon evolution of sulfur trioxide fumes. Remove the flame and allow the flask to cool slightly. Add 1 ml. of perchloric acid and continue heating until the solution has clarified. It may be necessary to add a few more drops of nitric acid at this point to complete the oxidation. Heat to the point where copious white fumes of sulfur trioxide appear and the perchloric acid has been destroyed. The final solution should be colorless, or, at most, a light straw color. Cool, add 40 ml. of redistilled water, and boil until copious white fumes of sulfur trioxide again appear. Continue heating for about 5 minutes to assure complete oxidation and elimination of perchloric and nitric acids. Cool, add about 10 ml. of redistilled water, and quantitatively transfer to a 100-ml. volumetric flask by washing with small portions of redistilled water until the volume is nearly 100 ml. Cool to room temperature and dilute to volume.

Alternate Method of Sample Preparation. This method is particularly advantageous for foods of high fat content.

Transfer an accurately weighed sample to a 300-ml. Kjeldahl flask, add 5 ml. of 30% hydrogen peroxide and 2 ml. of concentrated sulfuric acid, and heat gently until charring commences. Add 5 ml. more of the 30% hydrogen peroxide and continue heating until charring again occurs. Proceed as in the regular method, beginning with the addition of the nitric acid, a few milliliters at a time.

Procedure. Transfer a 25-ml. aliquot of the prepared solution to a 125-ml. separatory funnel and add exactly 5 ml. of concentrated hydrochloric acid. Add 1 ml. of 2% potassium persulfate and swirl the separatory funnel to ensure complete mixing. Add exactly 10 ml. of the 20% potassium thiocyanate reagent to develop the color, then add exactly 25 ml. of isobutyl alcohol and shake for 2 minutes. Draw off and discard the aqueous layer. Invert and slowly revolve the funnel to dislodge any water particles clinging to the walls and allow to stand for 10 minutes. Draw off the small amount of water which has separated from the alcohol, and transfer the alcohol layer to a dry 50-ml. Erlenmeyer flask. Immediately prior to reading the % transmission add a small amount (about 0.1 gram) of anhydrous sodium sulfate and agitate, to remove suspended particles of water from the alcohol extract. Read at 485 millimicrons, setting a reagent blank at 100% transmission. Obtain the micrograms of iron from a standard curve and convert to p.p.m.

If the color is too intense to read (in excess of 50 micrograms) repeat the determination, using a smaller aliquot of the prepared sample. As it is important that the volume ratio be kept constant, the difference in the aliquot size must be made up by the addition of redistilled water—for example, if a 15-ml. aliquot is used in place of the usual 25-ml., correct the difference in volume by adding 10 ml. of redistilled water.

Preparation of Standard Curve. Develop the color on increments of the working standard in the range of 0 to 60 micrograms of iron. A convenient formula to follow is:

5 ml. of concentrated hydrochloric acid
x ml. of standard
$(25 - x)$ ml. of redistilled water

From this point proceed exactly as outlined in the method, beginning with the addition of the potassium persulfate. Plot per cent transmission against the concentration on semilogarithmic paper.

Table II. Analyses of Foods

Product	Iron Content	Iron[a] Added	Total Iron Calculated	Total Iron Found
	P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*
Whole-kernel yellow corn	5.7	6.0	11.7	11.7
Peas	19.1	6.0	25.1	25.4
Tomatoes	6.5	6.5	13.0	12.5
Lima beans	14.6	3.7	18.3	18.2
Blackberry jam with added guava	7.3	5.5	12.8	12.5
Sliced bacon	7.3	4.8	12.1	11.9
Pork sausage meat	15.1	3.8	18.9	18.8
Wheat and soy egg noodles	39.7	10.0	49.7	49.8
Flour, enriched	27.7	10.0	37.7	37.7
Rice, converted, samples representing various stages in milling process				
Sample A	3.4	10.0	13.4	13.5
Sample B	8.8	10.0	18.8	18.8
Sample C	13.2	15.0	28.2	28.3
Sample D	16.5	10.0	26.5	26.5
Sample E	45.9	15.0	60.5	60.7
Patent flour, unenriched[b]	8.3	10.0	18.3	18.2
Patent flour, enriched with ferrum reductum[b]	30.6	5.0	35.6	35.5
Bread made from enriched flour[b]	32.8	5.0	37.8	37.7
Whole wheat flour[b]	37.3	10.0	47.3	47.5
Bread made from whole wheat flour[b]	39.5	5.0	44.5	44.5
Powdered whole milk	4.0	4.0	8.0	8.0
Powdered whole milk	4.1	6.0	10.1	10.6
Evaporated milk	4.0	2.0	6.0	6.0

[a] Iron added prior to digestion of sample.
[b] Submitted by Methods Committee of American Association of Cereal Chemists.

DISCUSSION

Contamination by fly ash so prevalent in industrial areas may be reduced to a minimum by acid digestion of the samples in Kjeldahl flasks. Thus the chief sources of contamination in the proposed method lie in the reagents. Sulfuric acid is the most frequent offender, but occasional lots of potassium thiocyanate and anhydrous sodium sulfate have proved unsatisfactory. In all cases reagent blanks should be run to determine the quality of new reagents. The author has found that, even in cases of reagent contamination, exact control of all volumes makes it possible to obtain satisfactory recoveries. In one instance, a badly

contaminated lot of sulfuric acid resulted in a blank of 75% transmission when compared to isobutyl alcohol set at 100%, but several points were checked on the standard curve with good agreement. Volumetric transfer pipets should be used throughout the method, so that the iron contamination due to a reagent will be kept absolutely constant throughout the blanks and the determinations. The addition of the redistilled nitric acid during the sample preparation need not be critically controlled, as this reagent will be iron-free.

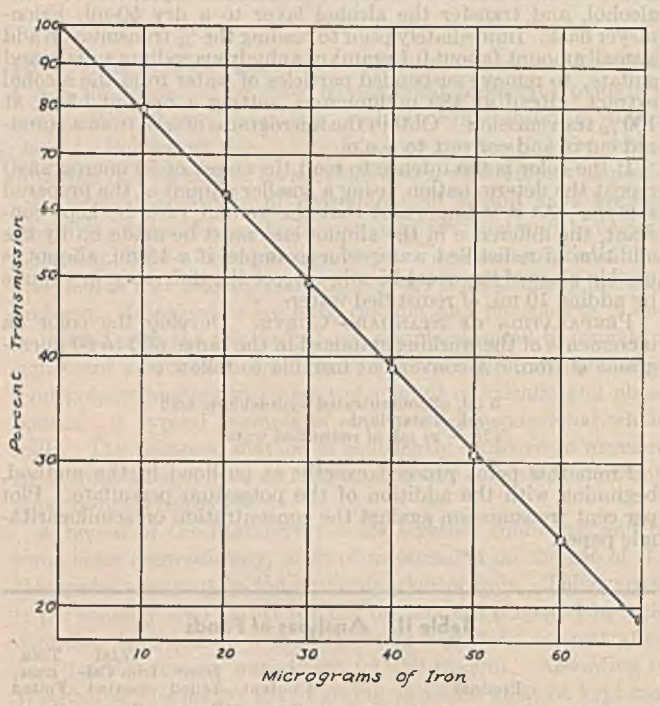

Figure 1. Standard Curve

The separation of the last traces of water from the isobutyl alcohol must be carefully controlled. Following the first separation the ratio of alcohol to water becomes very large, and if at this point more than gentle revolving of the flask is used a slight emulsion may occur. This will result in slow separation of water and may produce erratic results. Centrifuging will correct this difficulty. However, if the method is closely followed, clean separations may be easily obtained.

The quantity of anhydrous sodium sulfate added to the final extraction should be only sufficient to clarify the alcohol solution of suspended water. If more than the specified amount is added, the equilibrium between the dissolved water and the alcohol may be upset, resulting in the separation of a substantial portion of the dissolved water. With a few preliminary trials an analyst can easily estimate the amount to be added when using a small spatula, or, preferably, a scoopula.

The proposed method has proved entirely satisfactory when applied to a wide variety of food products (Table II). The recoveries have been better than ±0.5 p.p.m. As a further test of its reliability it has been submitted to three laboratories for additional checking and verification. One laboratory working with cereal products did not submit data obtained by the method but commented that it gave results in good agreement with values obtained by other methods (*1*). Two laboratories applied it to products that offer particularly difficult problems—beer with high phosphorus content and spray-dried whole milk and nonfat dry milk solids representing high-calcium foods. The results submitted by these two laboratories are shown in Table III.

The sensitivity of the method is exhibited by data obtained using two types of instruments. When the Coleman Universal spectrophotometer with 13-mm. cuvettes is used, the maximum range is from 0 to 70 micrograms of iron (Figure 1), representing a desirable working range of 3 to 50 micrograms. O'Malley (*6*) reports that using a Pfaltz and Bauer fluorophotometer as a filter photometer the maximum range was found to be from 0 to 30 micrograms of iron. This instrument uses a cuvette with a greater transmission path. The sensitivity of the Coleman Universal spectrophotometer may be correspondingly increased by using the larger cuvettes and the special cuvette carrier available for this instrument, as the volume of the isobutyl alcohol extract is sufficiently large to be read in the larger cuvettes. For general work the 13-mm. cuvettes are very satisfactory, as a wide range of iron concentration can be covered. For very low concentration where small samples are prepared, it is advantageous to use the larger cuvettes.

Table III. Analyses of Foods by Cooperating Laboratories

Product	Iron Content	Iron Added[a]	Total Iron Calculated	Total Iron Found
	P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*
Dry whole milk (spray)[b]				
Sample 1	3.5	8.0	11.5	12.2
Sample 2	1.8	8.0	9.8	9.2
Sample 3	6.8	10.0	16.8	16.4
Sample 4	3.3	8.0	11.3	10.8
Sample 5	7.6	..	..	..
Sample 5 (duplicate determination)	7.2	..	..	..
Sample 6	6.6	..	..	..
Sample 6 (duplicate determination)	6.4	..	..	..
Sample 7	4.0	..	..	..
Sample 7 (duplicate determination)	4.8	..	..	..
Nonfat dry milk solids (spray)[b]				
Sample 1	4.2	8.0	12.2	12.0
Sample 2	6.8	..	..	..
Sample 2 (duplicate determination)	7.2	..	..	..
Sample 3	6.8	..	..	..
Sample 3 (duplicate determination)	6.8	..	..	..
Beer[c]				
Sample A	0.24	0.80	1.04	1.04
Sample B	0.22	0.80	1.02	1.12
Sample C	0.22	0.80	1.02	1.08
Sample D	1.22	0.80	2.02	2.00
Sample E	0.38	0.80	1.18	1.10
Sample F	0.20	0.80	1.00	1.00

[a] Iron added prior to digestion of sample.
[b] Data submitted by American Dry Milk Institute, Laboratory Department, Chicago, Ill.
[c] Data submitted by Continental Can Co., Research Department, Chicago, Ill.

ACKNOWLEDGMENT

The author gratefully acknowledges his indebtedness to C. M. O'Malley and E. J. Baldi of the American Dry Milk Institute and Doris Grabenstetter and W. Stammer of the Continental Can Company for submitting the data on powdered milk and beer as shown in Table III. The suggestions submitted by these collaborators and J. S. Andrews of General Mills, Inc., have been of invaluable assistance in preparing this paper.

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Determining Ascorbic Acid in Large Numbers of Plant Samples

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A procedure for the determination of ascorbic acid in plant material is described which has been found useful in plant breeding and other plant research. It allows the rapid determination of ascorbic acid in a large number of samples of plant material with a satisfactory degree of accuracy.

IN THE course of plant breeding experiments the author desired to know the ascorbic acid content of many plants at different stages of development. Available methods for the determination of this substance in plants were too time-consuming to assure a reliable comparison of values from different sources inasmuch as changes might occur in the tissue while awaiting analysis. Hence it seemed imperative to devise a method which would permit the assay of many samples in the shortest possible time. This paper describes a procedure which has enabled the author and three student assistants to make 500 assays for ascorbic acid per day and to compare the results thus obtained with those of published methods.

It was obvious from early experiments that a more effective and rapid means of disintegrating plant tissue than hand grinding was necessary in order to gain speed in the assay as a whole. Hence a mechanical device (Figure 1) which makes possible the simultaneous disintegration of ten samples within 2 to 6 minutes, depending upon the texture of the tissue, was designed for the purpose and constructed in the machine shop of Michigan State College.

GRINDING MACHINE

As shown by Figure 1 this machine consists of a metal frame which supports a row of ten metal seats, each holding a porcelain cup 75 mm. high and 45 mm. in diameter. Porcelain pestles are attached to tapered chucks similar to those used in drill presses and are driven from a common horizontal shaft by means of belts and quarter-turn pulleys. Pressure on the pestle shafts can be varied by changing the position of the weights on the horizontal levers shown. Additional pressure can be obtained by holding down the levers singly or simultaneously with a cross bar.

While this investigation was in progress, Morell (7) published a method which possessed certain advantages but was still inadequate for the author's purpose. Morell made use of the so-called Waring Blendor for the maceration of plant samples. The efficiency of this Blendor was compared with that of the grinding machine described. The Blendor permits the use of much larger samples, and therefore reduces the error, but this advantage is lost if very small samples have to be disintegrated. Furthermore, the Blendor is ineffective for macerating certain types of plant material, such as seeds and fibrous tissue. These difficulties could not be overcome by the use of smaller Blendor cups as described by Davis (*3*) and Benne (*1*).

ASSAY

The Waring Blendor was used in most cases, but where it did not work well with a particular tissue the grinding machine was used.

A new method of handling very large samples, especially if a number of leafy plants should be examined together, has been successfully tested. A small portion of a sample is placed in the container with the liquid necessary for the extraction, and the machine is operated long enough to obtain a homogeneous mixture. The mixer is stopped, another part of the sample is added, and the procedure is continued until the entire sample has been disintegrated. In such a manner, voluminous samples weighing over 100 grams can be analyzed, but the extracts are rather viscous, and the filtration is therefore slow. If the extract appears to be too viscous, the amount of liquid should be increased. From the standpoint of accuracy a large sample is preferred. If the grinder is used, the weight of samples should not exceed 5 grams.

The titration is carried out with a rather concentrated solution of 2,6-dichlorophenolindophenol using a microburet. Flat dishes of glazed porcelain or sillimanite have been found extremely useful as containers. The small amount of extract is placed in their inner rim by means of a suitable pipet. The dish is held at an angle of approximately 60° (Figure 2) and gently rotated in the same plane through a short arc. By this means the liquid is moved enough to ensure its immediate mixture with the dye which is added drop by drop from the microburet. This is a very convenient procedure because the titration is completed rapidly and the end point is seen much more clearly than in any other way.

REAGENTS. Metaphosphoric acid, 20% stock solution. Dissolve 200 grams of metaphosphoric acid sticks in cold distilled water, filter, and dilute to 1 liter. Use one part of the stock solution and 9 parts of distilled water for extractions in the

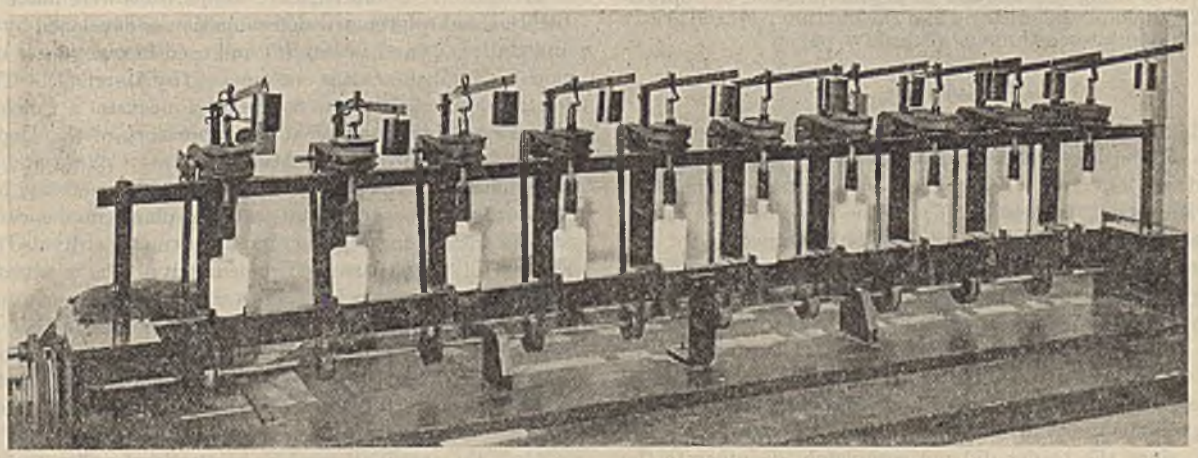

Figure 1. Grinding Machine for Simultaneous Disintegration of Ten Samples

Table I. Stability of 2% Metaphosphoric Acid Solution Stored at 4°

(As shown by ascorbic acid values of extracts)

HPO_3 Stored	Ascorbic Acid Found: Tomato juice, home made	Cauliflower, Purple Head, "curd"	Mustard, Tendergreen, leaves
Days	*Mg. per 100 grams of material used*		
0	12	67	166
5	13	67	166
10	12	67	167
15	12	67	162
20	12	67	165
30	12	67	160
40	11	64	148

Table II. Stability of Sodium 2,6-Dichlorobenzenoneindophenol in Aqueous Solution at 4°

[As compared by standardization after Menaker and Guerrant (5), expressed as percentage of value obtained on first day]

0	5 days	10 days	15 days	20 days	25 days
100	99.7	100	100	96.5	94.3

Table III. Ascorbic Acid in Fresh Plant Material

Plant Material	Ascorbic Acid Found[a]: Waring Blendor	Grinding machine	Hand grinding
	Mg. per 100 grams of fresh material		
Cabbage, wrapper leaves	81 ± 0.21[b]	81 ± 0.21	80 ± 0.15
Cauliflower, "curd"	89 ± 0.17	88 ± 0.19	92 ± 0.32
Gladiolus, leaves	316 ± 0.86	342 ± 1.38	322 ± 0.90
Mustard, leaves	156 ± 0.87	149 ± 0.86	150 ± 0.86
Pepper, green fruit	161 ± 0.45	161 ± 0.61	157 ± 0.82
Tomato, fruit	25 ± 0.38	24 ± 0.36	26 ± 0.38

[a] Means of 25 determinations.
[b] Standard deviation of mean.

mixer (2% solution). All concentrations can be stored in the refrigerator at 0° to 5° C. for 30 days (Table I).

Sodium 2,6-dichlorobenzenoneindophenol solution. Dissolve 200 mg. in 100 ml. of warm distilled water, filter, and dilute to 1 liter. When stored in the refrigerator the solution remains usable for at least 2 weeks (Table II).

Procedure with Blendor. One hundred milliliters of 2% metaphosphoric acid are pipetted into the dry glass container of the Blendor. The material to be examined (10 to more than 100 grams) is weighed to the nearest 0.1 gram, as suggested by Morell (7), and placed in the acid. The Blendor is operated for 2 to 5 minutes, depending on the kind and quantity of plant material. The liquid is filtered through qualitative filter paper (Green Nos. 488 and 704 were used in these experiments) into a 125-ml. Erlenmeyer flask. Aliquots of 0.1 to 5 ml. depending on the expected ascorbic acid content of the extract, are taken by pipet and titrated.

Procedure with Grinding Machine (Semimicromethod). Three milliliters of 20% metaphosphoric acid are pipetted into the grinding cups. The samples (different tissues or duplicates, as desired), weighed accurately to the second decimal, are placed in the cups, and a small amount of quartz sand is added. The samples are ground for 2 to 6 minutes, 27 ml. of distilled water are added to bring the solution to a concentration of approximately 2% metaphosphoric acid and the mixture is stirred briefly and gently and filtered through qualitative paper.

DISCUSSION

Ascorbic acid concentration in samples of various plant material as obtained by different methods of maceration was determined on a large scale (Table III). In all instances, when firm tissue was ground, the results of machine grinding were higher than those of hand grinding, which may be explained by the fact that hand grinding, when performed over a longer period, is tiresome and thus lowers efficiency. This is evident from the table inasmuch as cabbage, gladiolus, and pepper represented samples with comparatively firm tissue. Hand grinding gave lower yields of ascorbic acid in these cases if it was compared with the average of both machine grinding methods. In the case of gladiolus the Waring Blendor did not work satisfactorily, however, since the blades did not completely disintegrate the fibrous leaves.

In the comparison of the different ways of disintegrating plant material (Table III) it was necessary to use samples of different sizes according to the means of maceration employed, the samples prepared for the Blendor weighing 20 grams, those to be ground 2 grams. The small samples were selected as representative portions from the large ones, which were then reduced to the weight of 20 grams.

Filtration Versus Centrifugation. Centrifuging samples, as used in the standard method, slows up the process. It has been proved by numerous comparative tests that no losses of any significance occur if the extract to be tested is filtered through paper instead of being centrifuged. An extensive survey is given in Table IV on the basis of 500 tests made with various plant material. The plant tissue was disintegrated in the Waring Blendor and half of the extract was filtered through folded filter Green No. 488 while the other half was centrifuged for 10 minutes. The saving of time is clearly indicated by the fact that enough filtrate was available for titration in the average case within 3 minutes, whereas it took nearly 15 minutes to bring a centrifuged sample to that point. The results justify the abandonment of centrifugation in favor of filtration.

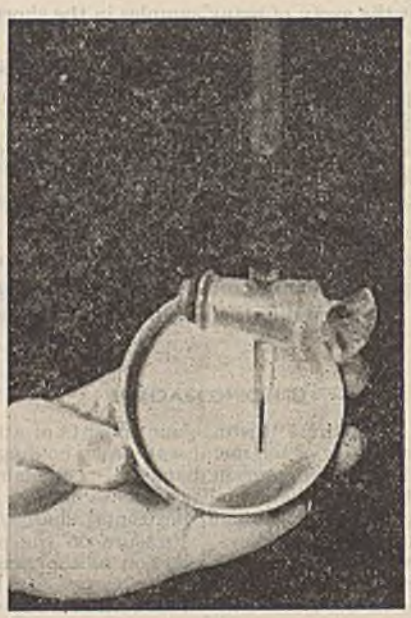

Figure 2. Glazed White Dishes for Microtitrations

Titration. Comprehensive comparisons were made between titration and colorimetric determination as developed by Mindlin and Butler (6) and Bessey (2) and used in many later modifications, as, for instance, the one proposed by Morell (7). The colorimetric determinations were made by means of a Coleman Universal spectrophotometer at the laboratory of the Department of Foods and Nutrition of Michigan State College and a Cenco photelometer at the Section of Agricultural Chemistry, Michigan Agricultural Experiment Station. The plant samples were macerated in the Waring Blendor, and the extract was divided in halves after filtration and examined immediately. The results are given in Table V. In some instances a significant difference appears to exist between the results obtained. However, higher readings were obtained in approximately as many cases with one method as with the other. It is concluded, therefore, that titration can, under certain conditions, well replace the more time-consuming colorimetric determination.

After the termination of these studies Loeffler and Ponting (4) published their adaptation of the photometric method of

Table IV. Ascorbic Acid in Filtered and Centrifuged Plant Extracts

Plant Material	Ascorbic Acid Found[a] Filtered	Centrifuged
	Mg./100 g. of material used	
Basswood, leaves (July)	70 ± 0.43[b]	71 ± 0.45
Beans, string, fresh		
Variety 1	16 ± 0.30	16 ± 0.36
Variety 2	10 ± 0.21	10 ± 0.23
Variety 3	10 ± 0.13	10 ± 0.15
Variety 4	26 ± 0.30	26 ± 0.26
Variety 5	15 ± 0.23	15 ± 0.21
Beans, string, dehydrated		
Variety 6	16 ± 0.15	16 ± 0.10
Variety 7	9 ± 0.27	9 ± 0.22
Variety 8	9 ± 0.18	9 ± 0.13
Variety 9	17 ± 0.23	17 ± 0.18
Variety 10	38 ± 0.35	38 ± 0.20
Cabbage, heads		
Variety 1	47 ± 0.18	48 ± 0.29
Variety 2	38 ± 0.22	38 ± 0.13
Variety 3	32 ± 0.22	34 ± 0.18
Variety 4	46 ± 0.20	47 ± 0.22
Variety 5	37 ± 0.23	37 ± 0.16
Variety 6	37 ± 0.16	36 ± 0.15
Variety 7	33 ± 0.16	34 ± 0.16
Variety 8	46 ± 0.22	46 ± 0.22
Variety 9	50 ± 0.21	50 ± 0.15
Cabbage, leaves of flowering plant		
Sample 1	129 ± 0.43	129 ± 0.43
Sample 2	162 ± 0.33	159 ± 0.87
Cauliflower, "curd"		
Sample 1	77 ± 0.23	77 ± 0.23
Sample 2	103 ± 0.50	103 ± 0.53
Cauliflower, leaves	74 ± 0.58	74 ± 0.58
Dandelion, flowers	21 ± 0.30	21 ± 0.33
Kohlrabi, edible portion	75 ± 0.23	75 ± 0.22
Kohlrabi, leaves	146 ± 0.65	145 ± 0.53
Mustard leaves (grown in greenhouse)		
Sample 1	76 ± 0.40	75 ± 0.34
Sample 2	73 ± 0.29	72 ± 0.38
Sample 3	65 ± 0.23	65 ± 0.31
Sample 4	48 ± 0.35	49 ± 0.16
Sample 5	99 ± 0.37	98 ± 0.34
Pepper, green fruit		
Sample 1	157 ± 1.08	160 ± 1.09
Sample 2	257 ± 1.65	257 ± 1.66
Pepper, leaves		
Sample 1	98 ± 0.81	98 ± 0.76
Sample 2	167 ± 1.48	167 ± 1.37
Radish, tops (grown in greenhouse)	82 ± 0.55	80 ± 0.31
Red maple, blossoms	155 ± 1.61	156 ± 1.50
Ribes aureum, blossoms	61 ± 0.62	60 ± 0.57
Tomato, fruit		
Variety 1	17 ± 0.37	17 ± 0.54
Variety 2	23 ± 0.42	23 ± 0.51
Variety 3	31 ± 0.38	30 ± 0.27
Variety 4	35 ± 0.33	35 ± 0.25
Variety 5	29 ± 0.34	28 ± 0.26
Variety 6	28 ± 0.39	29 ± 0.39
Variety 7	19 ± 0.31	19 ± 0.20
Variety 8	24 ± 0.20	24 ± 0.15
Variety 9	25 ± 0.26	24 ± 0.21
Variety 10	23 ± 0.27	24 ± 0.31

[a] Means of 10 determinations.
[b] Standard deviation of mean.

ascorbic acid determination. They emphasized the fact that "some analysts still use the erratic titration with an unbuffered acid" and mentioned 5% sulfuric acid plus 2% metaphosphoric acid in particular, as used by Mack and Tressler. They showed graphically the rate of fading of the indophenol reagent as caused by various acids in the absence of ascorbic acid. Although the diagram indicated that metaphosphoric acid does not cause much fading, it was considered appropriate to examine the conditions which exist during titration with an unbuffered acid.

Solutions of ascorbic acid in 1, 2, 3, 5, and 10% metaphosphoric acid, in 5% sulfuric plus 2% metaphosphoric acid, and in 0.4% oxalic acid, as recommended by Loeffler and Ponting (*4*), were prepared and tested (Table VI). Three kinds of plant material, tomato fruits, tomato leaves, and leaf lettuce, were extracted with equal amounts of 1, 2, 3, 5, and 10% metaphosphoric acid, 5% sulfuric acid plus 2% metaphosphoric acid, and 0.4% oxalic acid. The plant material was cut in small pieces, mixed thoroughly, and then divided in equal portions which were blended with the different acid solutions in a Waring Blendor. Table VII shows the ascorbic acid values found in these samples; 5 and 10% metaphosphoric acid caused some fading of the dye but the only serious case was represented by sulfuric plus metaphosphoric acid where a very considerable fading occurred. This confirms Loeffler and Ponting's findings. For unknown reasons there was much less interference of the strong acids when certain plant material was used. The conclusion can be drawn that 1 to 3% metaphosphoric acid can be used for titration with no loss of ascorbic acid due to insufficient extraction and without the danger of destruction of the dye by the extracting acid.

In addition, the action of several concentrations of metaphosphoric acid on the indophenol dye was observed. Fading of the dye could be seen in 10% metaphosphoric acid if the solutions were mixed and allowed to stand for 2 to 3 minutes. However, after 3 minutes the degree of fading was not sufficient to cause an error of more than 3% in titration of ascorbic acid present in the metaphosphoric acid. This is immaterial in view of the fact that titrations as used in the procedure described require no more than an average of one minute each. Furthermore, much weaker concentrations of metaphosphoric acid are used in titrations with dichlorobenzenoneindophenol.

It is concluded that titrations as used in the procedure presented can be accomplished safely and reliably with unbuffered solutions.

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Table V. Ascorbic Acid Values of Fresh Plant Materials

Plant Material	Ascorbic Acid Found[a] Titration	Colorimetry
	Mg./100 g. of material used	
Cabbage, Golden Acre, sections of head	30.9 ± 0.39[b]	30.5 ± 0.01
Cabbage, Golden Acre, wrapper leaves	81.7 ± 0.16	82.0 ± 0.09
Cabbage, Wisconsin Hollander, sections of head		
Sample 1	36.6 ± 0.13	38.2 ± 0.07
Sample 2	46.2 ± 0.09	45.1 ± 0.01
Sample 3	37.1 ± 0.09	50.3 ± 0.12
Sample 4	42.0 ± 0.10	44.1 ± 0.01
Cabbage, Wisconsin Hollander, wrapper leaves		
Sample 1	92.6 ± 0.07	92.7 ± 0.01
Sample 2	91.8 ± 0.08	89.4 ± 0.01
Sample 3	95.5 ± 0.06	97.6 ± 0.01
Sample 4	99.1 ± 0.07	93.1 ± 0.01
Pepper, Harris King of the North, green fruit		
Sample 1	127.6 ± 0.10	123.6 ± 0.01
Sample 2	156.9 ± 0.12	162.5 ± 0.10
Sample 3	141.4 ± 0.14	144.0 ± 0.02
Sample 4	138.2 ± 0.12	141.4 ± 0.12
Gladiolus, Orange King, leaves		
Sample 1	374.9 ± 0.21	360.7 ± 0.54
Sample 2	323.1 ± 0.62	312.2 ± 0.35
Sample 3	327.4 ± 0.32	335.9 ± 0.30

[a] Means of 10 determinations.
[b] Standard deviation of mean.

Table VI. Titration Values of Ascorbic Acid Dissolved in Metaphosphoric and Other Acids

Acid Concentration	Ascorbic Acid Values[a]
1% HPO_3	100
2% HPO_3	100
3% HPO_3	100
5% HPO_3	102
10% HPO_3	104
5% H_2SO_4 + 2% HPO_3	125
0.4% $(COOH)_2$	100

[a] Expressed as percentage of value obtained in 2% HPO_3.

Table VII. Titration Values of Ascorbic Acid

Extracting Acid	Greenhouse Tomatoes (Immature Fruits)	Tomato Leaves	Greenhouse Leaf Lettuce[a]
	Mg. per 100 grams of material used		
1% HPO_3	12	22	12
2% HPO_3	12	22	14
3% HPO_3	12	21	13
5% HPO_3	12	24	13
10% HPO_3	13	25	13
5% H_2SO_4 + 2% HPO_3	13	28	15
0.4% $(COOH)_2$	11	21	14

[a] Average values of two varieties.

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BOOK REVIEW

Laboratory Experiments in Biological Chemistry. *James B. Sumner and G. Fred Somers.* 169 pp., 17 figures. Academic Press, Inc., New York, N. Y., 1944. Price, $2.60.

This volume was written as a laboratory guide for a course given to students of biochemistry at Cornell University. In the words of the author, "it is intended to be . . . general and to provide fundamental training in laboratory biochemistry to students in any field of study". It consists for the most part of 252 exercises which are largely written in the imperative, and with a minimum of detail and discussion.

In examining this small volume, the reviewer was most impressed by the amount and diversity of material which was compressed in a small space. It was obviously not intended to cover every phase of biochemistry, being most deficient in clinical procedures. Short of the larger reference books, it gives the most diverse treatment of the chemical phases of the subject that the reviewer has seen.

The greater part of this book is devoted to instructions for qualitative testing of many types of biological materials. It even includes more or less systematic procedures for identifying unknown fats, carbohydrates, and proteins singly and in mixtures. From the standpoint of qualitative biochemistry alone, this little volume should find a useful place on the biochemist's bookshelf.

The quantitative aspects of the field have been treated much less completely than the qualitative. Many of the standard analytical procedures are given in sufficient detail for routine performance, and most common types of biochemical analysis are included. It is unfortunate that most of the quantitative methods are old ones which are widely used but do not represent the best analytical procedures available at present. In this respect, this book will not serve to advance the progress of quantitative analysis by biochemists. A large proportion of the methods given are those of Folin, in original or modified form. Phosphate determination is made by the old method of Fiske and Subbarow and the Allen modification. No mention is made of the very advantageous procedure of Berenblum and Chain. Similar omissions might be noted for most of the quantitative procedures.

An unusual feature of this book as compared with others of the same type is its emphasis on safety and proper practice. This is a field which has been largely neglected in college textbooks and in teaching, in spite of the example of industry, where safety has been a primary consideration in many instances. A stronger trend in this direction is greatly to be desired.

As is always to be expected in a first edition, some minor deficiencies and inaccuracies are occasionally apparent. On page 23, it is not at all clear how a student is to "observe the absorption band in the red at 635 mμ". No mention is made of the use of any dispersion device for the purpose. On page 87, the description of the xanthoproteic test does not include any indication of the group in the protein molecule which gives the reaction, except for the statement: "Benzene compounds react with nitric acid to form yellow nitro compounds. Picric acid is an example." The other protein tests uniformly list the group responsible for the test. The statement on page 12, that "centrifuge tubes that are heated for the purpose of drying them become very brittle and are likely to break while being centrifuged", must seem surprising to one familiar with glass behavior. It would certainly have been better to make the statement accurate than to pass the phenomenon of strain formation off as brittleness.

The treatment of electrometric determination of pH is too nearly complete to force the student to consult a better reference, but is inadequate as a complete explanation in itself. Similarly, the treatment of colorimetry and the "photoelectric colorimeter" leaves something to be desired. For example, the discussion of Beer's law on page 71 omits the very important fact that this law was derived for monochromatic light and is not applicable to polychromatic radiation. This omission is even more serious in the discussion on page 37, of the Duboscq colorimeter, which employs white light and approximates obedience to Beer's law over short ranges only. This fact was tacitly recognized in the statement "the concentration of the unknown cannot be determined accurately in many cases if it differs too greatly from the concentration of the standard". On page 73, the injunction to use test-tube type absorption cells which "are of uniform thickness all the way around and are perfectly round" will certainly discourage the conscientious student. Studies of hundreds of such tubes have failed to produce even one which meets the above specifications. It would have been more nearly in the realm of possibility to require that tubes be matched with respect to rotation and used in the positions in which such matches were found.

Typography, make-up, and binding of the book are very satisfactory. The printing is clear and remarkably free of typographical errors. In spite of the fact that this volume does not accomplish all that might be desired in a general laboratory manual, it is a valuable addition to the biochemical literature which can serve as the basis for a very effective laboratory course in biochemistry.

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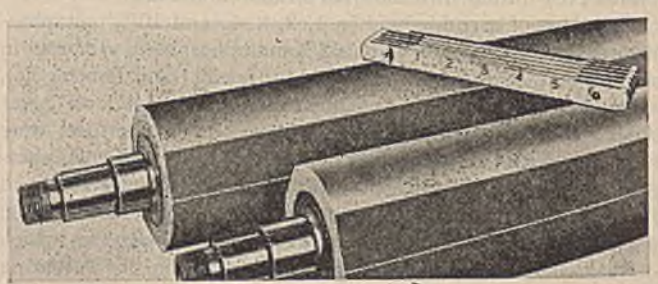

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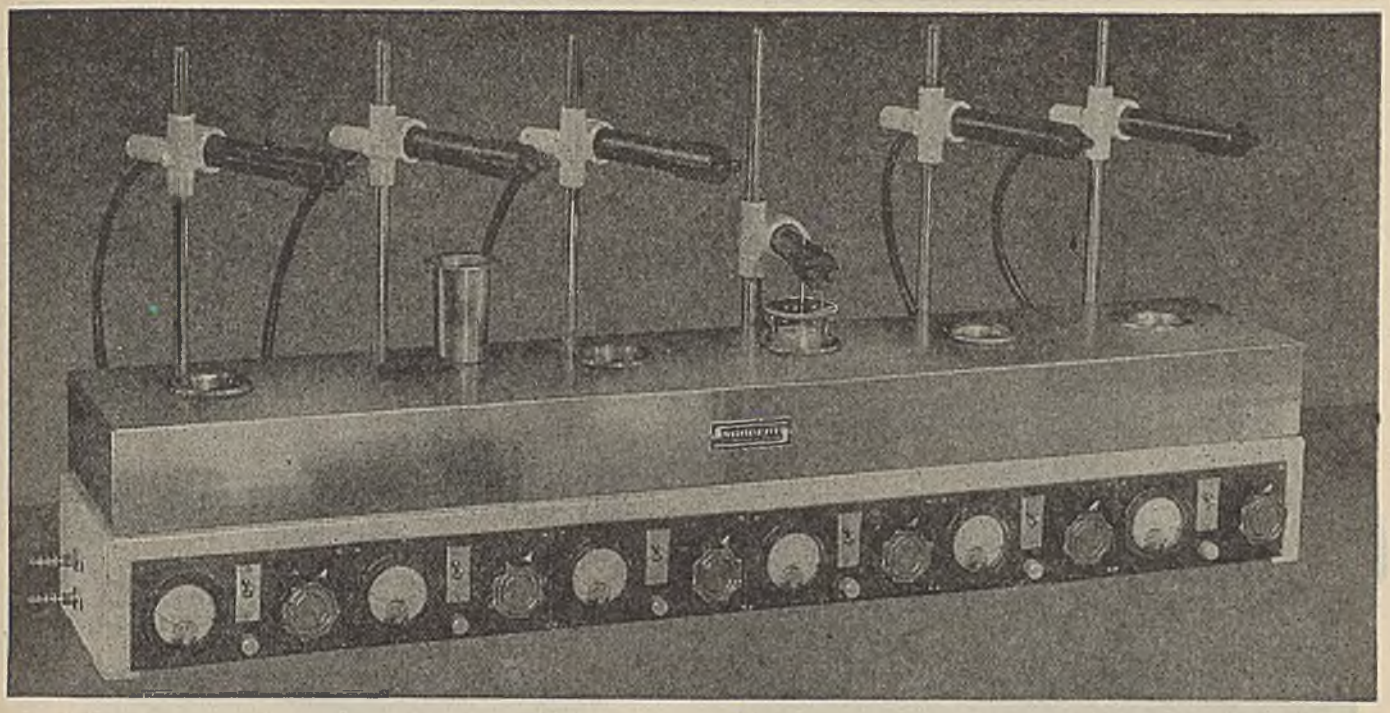

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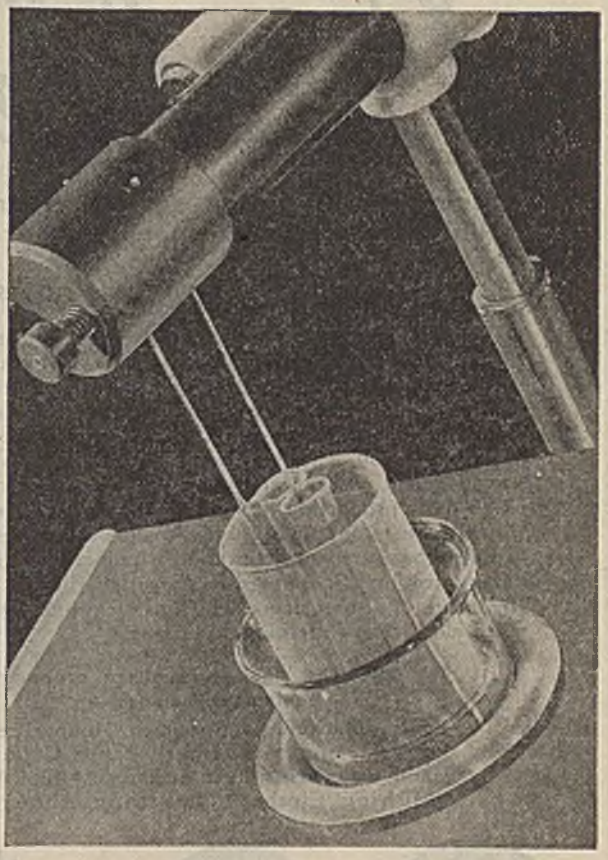

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